PRESENT THE

LAKE WANAKA VETERINARY CONFERENCE 2010
"ONCOLOGY IN THE SNOW"

CONFERENCE PROCEEDINGS

Edgewater Resort, Lake Wanaka, New Zealand
August 9 to August 13, 2010

Speakers:
Dr Veronika Langova & Dr Martin Havlicek

LECTURE TIMETABLE

Monday 9th August
7.30 am - 9.30 am
Why do we treat cancer? Staging, diagnostics and more
Dr Veronika Langova

5.00 pm - 7.00 pm
How much is enough? Principles of biopsies
Anaesthesia and pain control for our patients
Dr Martin Havlicek

Tuesday 10th August
7.30 am - 9.30 am
Anaemia in practice. Is it regenerating? Why not?
Dr David Collins

5.00 pm - 7.00 pm
What do we need to know about chemotherapy? When and why?
Dr Veronika Langova

Wednesday 11th August
7.30 am - 9.30 am
Transfusions - when do we give blood? plasma? How much and how fast?
Dr Karina Graham

5.00 pm - 7.00 pm
Oral tumours & Intrathoracic tumours
Dr Veronika Langova & Dr Martin Havlicek

Thursday 12th August
7.30 am - 9.30 am
Haemostasis and coagulopathies made easy
Dr Anna Byron

5.00 pm - 7.00 pm
The new and the old about lymphoma in dogs and cats
Dr Veronika Langova

Friday 13th August
7.30 am - 9.30 am
Bone tumours - what options do we have?
Dr Martin Havlicek

5.00 pm - 7.00 pm
The great imitators - mast cell tumours
Dr Veronika Langova

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LAKE WANAKA VETERINARY CONFERENCE 2010

SPEAKERS

Dr Veronika Langova
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Veronika graduated in Brno, Czech Republic in 1992. She was admitted to the Australian College of Veterinary Scientists as a Member in Small Animal Medicine in 2000. In 2005, she attained Fellowship in Small Animal Oncology after completing her residency at Animal Cancer Care in Brisbane. During 2006-2007, she joined the team of oncologists at the Colorado State University as an Associate Professor. She spent the last a year working at the Queensland Veterinary Specialists and Veterinary Specialist Services in Brisbane before moving to the Veterinary Specialist Centre in 2008..

Veronika is a member of Veterinary Cancer society, the Postgraduate Foundation, Australasian Association of Veterinary Diagnostic Imaging and the Australian Veterinary Association. She is interested in cancer research and has devoted time to Engeneic Pty Ltd as a clinical trial coordinator, researching on multi-drug resistance and targeted chemotherapy delivery. She enjoys hiking, mountain biking, skiing, swimming and windsurfing in Australia in her free time.

Dr Martin Havlicek
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Martin graduated from the University of Veterinary and Pharmacological Sciences in Brno, Czech Republic, in 1992. He came to Australia in 1994 and was working with Advanced Anaesthesia Specialists from 1997 - 2000. He completed an internship in small animal surgery at the Animal Referral Hospital in Strathfield in 2000 and was admitted to the Australian College of Veterinary Scientists as a Member in Small Animal Surgery in the same year. He then worked in a private practice in Brisbane, before taking up the position as a clinical

instructor at the University of Queensland. In 2006, he completed a Residency training program in Small Animal Surgery at the Brisbane Veterinary Specialist Centre. He then travelled to the UK, Wey Referrals and The Animal Health Trust as a soft tissue and oncology surgeon during 2006 - 2007. He obtained his Fellowship in Small Animal Surgery of the Australian College of Veterinary Scientist in 2009.

He is a member of the Veterinary Orthopaedic Society, Veterinary Endoscopy Society and the Veterinary Cancer Society

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Dr Veronika Langova
Oncology

It is fairly routine medical practice for veterinarians to treat domestic small animal pets for diabetes mellitus, congestive heart failure and chronic renal failure. These animals receive treatments and drugs that ameliorate some of the signs of their diseases and this may prolong their survival.

Mention treating cancer and many pet owners and veterinarians reel back in horror despite the fact that cancer is the only curable chronic disease. Not all cancers are rapidly progressing, life threatening entities. The approach to cancer treatment varies from a radical approach seeking cur, to palliation, which allows the patient to live a good quality of life with their disease.

Approximately 50% of people with serious cancers can be cured of their disease. For certain types of cancers in domestic pets this figure is much higher. Dogs with certain soft tissue sarcomas can have a 90% chance of cure. Although lymphoma in dogs and cats is rarely cured up to 90% go into complete remission for an average of approximately 12 months with treatment and only 10-20% experience toxicity from treatment and this toxicity is short lived. There are many other examples of successful cancer outcomes.

Cancer is recognized to be the leading cause of death in our companion animals. Surgery, radiotherapy and chemotherapy remain for the time being the standard treatment modalities in the fight against cancer. The golden rules of oncology remain the same: Goal is to establish histological diagnosis, determine the stage of the disease and assess any concurrent health problems of the patient.

Technology is moving fast and its new developments are improving the diagnostics as well as treatments able to be used in oncology. For example in diagnostics, the use of monoclonal antibodies to immunophenotype tumours like lymphoma has been shown to be prognostic value. Other technologies as assessment of DNA content by flow cytometry will become used to type and grade cancer in the future. Increased availability of ultrasound and other advanced imaging modalities as computed tomography (CT) and magnetic resonance imaging (MRI) had improved our ability to detect and determine the true extent of the disease as well as plan our surgery.

It is time for all veterinarians treating small animal patients to reassess their approach to treating cancer. Our patients deserve it. Our clients demand it. There have been great advances in treatment opportunities and alternatives.

Why treat cancer? Why not?

DIAGNOSIS

Accurate interpretation of a properly acquired biopsy specimen is probably the most important step in cancer management. *Pathology is everything!* Not only will the biopsy provide the means to establish the diagnosis but also it allows prediction of biologic behavior. This provides a basis for therapeutic alternatives such as the type and extent of treatment. Basically all masses should be histologically evaluated before or after removal. *If it's worth taking off it's worth looking at!* Unfortunately the biopsy is often performed too perfunctorily (or not at all!) which almost invariably leads to serious problems in patient management. The clinician must keep the three basic principles of oncology *("biopsy, biopsy, and biopsy")* in focus but must not be so single minded as to perform a biopsy in a fashion that jeopardizes the patient's prognosis or quality of life or that increases the risks of the definitive procedure. For example, if a lesion on an extremity is biopsied through a transverse skin incision as opposed to a longitudinal one, the tumour may not be able to be removed with a limb preserving surgery because many tissue compartments have been contaminated by the biopsy. Remember, the entire biopsy tract must be removed *en masse* with the tumour with any definitive surgery. In this example this may only be possible by otherwise unnecessary limb amputation. The extent of problems from biopsy are not known in veterinary medicine, however, the Musculoskeletal Tumour Society published a report from information on 329 people with newly diagnosed soft tissue and bone malignancies. There were major errors in the diagnosis in 18.2%, non-representative or technically poor biopsies in 10.3%, problems in the skin, soft tissue or bone of the biopsy wound in 17.3%, and the optimum treatment had to be altered as a result of problems related to the biopsy in 18.2%. An unnecessary amputation was performed as a result of problems related to biopsy in 4.5% and the prognosis and outcome was considered to be adversely affected in 8.5%. This does not mean biopsy should be avoided; the recommendation based on these data is that the biopsy should be planned as carefully as definitive surgery. Biopsy is not known to increase the risk of systemic spread of cancer (metastasis), however tumour cells can spread within the biopsy surgical field or into body cavities increasing the risk of local tumour spread or tumour implantation. Example is transabdominal biopsy of transitional cell carcinoma of the bladder of splenic heamangiosarcoma

Many variations in technique and equipment for biopsy procedures are described in the veterinary literature, but the common goal is to procure enough neoplastic tissue to establish an accurate diagnosis. Many biopsy techniques could be used on any given mass. Which procedure to use will be determined by specific goals for the case, site of the mass, equipment available, general status of the patient and personal preference and experience.

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INTERPRETATION OF RESULTS

The pathologist's job is to determine: 1) tumour vs. no tumour, 2) benign vs. malignant, 3) histological type, 4) grade (if applicable), and 5) margins (if excisional). Making an accurate diagnosis is not as simple as putting a piece of tissue in formalin and waiting for results. Many pitfalls can take place to render the end result inaccurate. Potential errors can take place at any level of diagnosis and it is up to the clinician in charge of the case to interpret the full meaning of the biopsy result. If the biopsy result does not correlate with the clinical scenario, several options are possible:

1. Call the pathologist and express your concern over the biopsy result. This exchange of information should be helpful for both parties and not looked upon as an affront to the pathologist's authority or expertise. It may lead to:
    a. Re-sectioning of available tissue or paraffin blocks
    b. Special stains for certain possible tumour types (e.g. toluidine blue for mast cells)
    c. A second opinion by another pathologist.
2. If the tumour is still present in the patient, and particularly if widely varied options exist for therapy, a second (or third) biopsy should be performed.

A carefully performed, submitted and interpreted biopsy may be the most important step in management and subsequent prognosis of the patient with cancer. All too often tumours are not submitted for histological evaluation after removal because "the owner didn't want to pay for it".

Biopsies should not be an elective owner decision. *Biopsy is as fundamental to proper case management as closing the skin after ovariohysterectomy.* The charge for submission and interpretation of the biopsy should be included in the surgery fee if need be but the biopsy must be done. *Because of increasing medico legal concerns, it is not medical curiosity alone that mandates knowledge of tumour type.*

IMMUNOHISTOCHEMISTRY

Immunohistochemistry has been used in human medicine for many years, but only recently has become useful diagnostic tool in veterinary medicine. The use of immunohistochemistry in veterinary medicine is continuing to increase and plays an important role in tumour identification and the prognosis.

Immunohistochemistry uses antibodies directed against specific cellular antigens that are markers of specific cell type. The antibodies are linked to dyes that are visualized under microscope. Commonly used immunohistochemical stains are those for intermediate filaments such as vimentin for mesenchymal cells, cytokeratin for epithelial cells, desmin or actin for muscle cells. Other proteins or secretory products that may be identified are factor VIII antigen for endothelial origin tumours, insulin or thyroglobulin. For lymphoid tumours, determination of CD3 (marker of T-cell) or CD 79a (marker of B-cell) origin has been shown to be an important prognostic indicator, because remission rate as well as length of remission in T-cell lymphoma patient is significantly shorter then in B-cell lymphoma cases.

Important fact to realize is that immunohistochemistry does not distinguish normal from neoplastic tissue; it only identifies the cell of origin.

SPECIAL STAINS

Toluidine blue is most commonly used to identify mast cell tumours, Periodic acid Schiff (PAS) can help to identify mucin or other secretions associated with epithelial tumours. AgNOR staining has been used to determine degree of malignancy of mast cell tumours.

TUMOUR STAGING

Cancer is the only curable chronic disease. Surgeons can cure cancer by finding a patient without metastatic disease, correctly identifying the cancer tissue and cutting completely around it without leaving or spilling any cancer cells in the body. Besides a degree of luck and good judgment for this event to transpire, the patient must be adequately *STAGED*. Staging is the technique of breaking tumours up into categories. The cancer therapist must know, therefore, to which "category" the patient's tumour belongs. Staging is a way of separating "early cancer" from "late cancer" or "advanced cancer". In this sense, a time related progression of the disease is described. The implication is that survival rates are higher for patients with localized cancer compared to those with distant spread of disease. This is important information for deciding the proper surgical dose, or whether a cure is possible and may influence a pet owner's willingness to treat. Staging must be done in a systematic, reproducible way. A detailed description of the World Health Organization T N M Classification of Tumours in Domestic Animals is beyond the scope of this discussion. Rather, I want to address staging in the context of "surgical oncology thinking". Readers are directed to two references in particular for more detailed information about staging:

1. UICC TNM Classification of Malignant Tumours, Ed., M. Harmer, 3rd ed., Geneva, 1978.
2. Owen LN. World Health Organization T N M Classification of Tumours in Domestic Animals, In Clinical Veterinary Oncology, Withrow SJ and MacEwen EG Eds., JB Lippincott Co., Philadelphia, 1989, pp 448-189.

THE OBJECTIVES OF A TUMOUR STAGING SYSTEM

1. To help plan appropriate treatment
2. As a prognostic tool
3. To assist in evaluation of treatment results
4. To standardize information to enable comparison of outcome from different trials at different institutions
5. To contribute to the continued investigation of animal cancer
6. To help establish comparative models for human cancer

The TNM system has been proposed as a way to meet these objectives.

THE TNM SYSTEM

T - represents an assessment of the extent of the primary tumour.

N - represents an assessment of the involvement of the regional lymph nodes

M - is an indicator of whether metastatic disease is detectable

The extent of malignant disease is then represented by adding numbers e.g. T1, T2...N0, N1...M0, M1...etc. This can be made even more sophisticated by adding a lower case letter to the T classification to represent the presence or absence of fixation to surrounding tissue, or ciphers (- or +) to the N classification to denote the absence or presence of histological confirmation of tumour extension to an enlarged lymph node

STAGE GROUPING

The TNM classification provides a means for precise recording of the apparent extent of disease. For identifying statistically meaningful differences in survival probabilities cases are generally divided into clinical groups. Unfortunately the tumour staging in the current system is not always applicable to the clinician because it may not truly reflect a meaningful prognostic guide in every tumour type or location. We still need a modification of this system, which has largely been an adaptation form a human tumour classification system, so that tumour classification correlates with survival probability. Even given these shortcomings it is vital that the clinical oncologist know about tumour staging and the tools available to perform this task.

THE PRIMARY TUMOUR

The histological type and (in many instances) the histological grade of tumour are of major importance to selecting the proper surgical technique. Virtually all external masses should have a minimum of cytological evidence of benign versus malignant disease. Preferably, a biopsy should be performed and evaluated BEFORE the definitive surgical treatment. The principles of biopsy are presented elsewhere in this seminar series but since *Pathology is everything - all our efforts, expertise, judgment and evaluations hinge on obtaining a correct histological diagnosis*; it is warranted to stress the importance of biopsy. **IT IS IMPERATIVE THAT YOU KNOW WHAT YOU ARE TREATING!**

You also need to know where the tumour is or rather the anatomical extent of the local tumour. This is the **T** classification. Tools for assessing this include: physical examination, standard radiographic techniques, ultrasonography, contrast studies (angiography, cystography, gastrography, pneumoventriculography, myelography etc.), endoscopy, tomography, computed tomography (CT), magnetic resonance imaging (MRI), nuclear scintigraphy, single photon emission computed tomograph (SPECT), thermograph and on and on. Some of the more sophisticated tests are very useful in certain anatomical sites and for various tumours but in other situations are at the very least "over kill" and lend nothing to making a decision for appropriate treatment. Examples of some of these situations will be discussed.

THE REGIONAL LYMPH NODE

Assessment of the involvement of the regional lymph node is the **N** part of the staging formula. A great deal of controversy surrounds the surgical management of regional lymph nodes draining the primary tumour site. As a general rule, epithelial cancers are more likely to metastasize to lymph nodes than are mesenchymal cancers. However, any enlarged regional lymph node requires investigation. Lymphadenomegaly may be from metastasis of cancer (firm, irregular and sometimes fixed to surrounding tissue) or from hyperplasia and reactivity to various tumour factors, infection, or inflammation. The former cause is a poor prognostic sign and the latter may be a beneficial host response. Enlarged lymph nodes as a result of cancer metastasis and invasion are generally uniformly effaced by tumour cells and can often be diagnosed by fine needle aspiration. Positive lymph nodes usually are a sign of impending systemic metastasis. Lymph nodes should be removed under two general circumstances:

1. If the lymph node is positive for cancer and not fixed to surrounding normal tissues, it may be possible to remove the node with some therapeutic intent. Frequently however, many lymph nodes drain a primary tumour site (e.g. oral cavity) and lymphadenectomy is incomplete (e.g. neck dissection). Although it is usually not practical, removal of the primary tumour, intervening lymphatic ducts and draining lymph node has been recommended (*en bloc* resection). *En bloc* resection may be possible for a malignant toe tumour with metastasis to the popliteal lymph node, but is usually only accomplished with amputation. Few other anatomic sites are routinely amenable to this therapy.

2. Normal appearing lymph nodes which are known to drain a primary tumour site should be randomly sampled (biopsy or cytology) to gain further staging information. This is particularly important if adjunctive treatment decisions (irradiation or chemotherapy) would be predicated on confirmation of residual cancer. Intrathoracic or intraabdominal lymph nodes are perhaps most crucial since they are not readily accessible to follow-up examination.

Lymph nodes are <u>not</u> removed under two general circumstances:

1. Lymph nodes in critical areas (retropharyngeal, hilar, mesenteric) which have eroded through the capsule and become adherent (fixed) to surrounding tissues cannot be curatively removed without serious harm to the patient. They are best biopsied and left alone or treated with other modalities. The occasional exception is metastasis of limb and foot tumours to prescapular and popliteal lymph nodes, which can be removed with amputation (radical *en bloc* resection).

2. Prophylactic removal of "normal" draining lymph nodes or chains of lymph nodes (as opposed to sampling for stage) is of no benefit and may be harmful. Regional lymph nodes may in fact be the initiator of favorable local and systemic immune responses and elective removal has been associated with poor survival in certain human cancers.

METASTASIS

In veterinary practice the assessment of the **M** classification is the evaluation of good quality, properly positioned thoracic radiographs. In some cases abdominal radiography may be important, such as for mast cell tumours. Thoracic radiographs should be taken with the animal conscious. Anesthetized animals very rapidly develop atelectasis in the lung lobes of the dependant hemi thorax. This makes identification of soft tissue dense lung metastasis inaccurate. It is advisable to take both lateral views to make a complete evaluation of the pulmonary tissue. Tumours in the uppermost lung fields will be outlined by stark contrast to the air filled lung whereas the dependant lung has more blood perfusion and there is a soft tissue to fluid density contrast making identification of lung nodules difficult. Nodules may even "disappear" on contralateral views. It is important to perform this part of the staging process as accurately as possible because in most cases, metastatic disease has a serious negative impact on survival. Even with the best technique, small lung nodules (less than .75 to 1 cm in diameter) may be missed with plain radiography. Fluoroscopy and lung CT may help improve the sensitivity. Nuclear scintigraphy is not uniformly a good tool for identifying metastatic disease in soft tissue but with the appropriate radiopharmaceutical it is an extremely sensitive study for identifying bone metastasis. Unfortunately nuclear bone scans are not selective and many disorders of bone may mimic bone metastasis (tooth abscess, arthritis, healing fractures etc.).

What do you do with the **M1** case? This is an appropriate time to talk about palliation. Palliative surgery is an attempt to improve the quality of the patient's life (pain relief or improved function) but not necessarily the length of the patient's life. This type of surgery requires very careful consideration of the expected morbidity of the procedure versus the expected gain to the patient and the client. In essence, it comes down to a decision of when to give up. One of the most difficult decisions in surgical oncology is the decision <u>not</u> to operate. Treatment of any kind should never be worse than no treatment.

Certain situations do exist, however, where palliative surgery may be beneficial. If an infected and draining mammary tumour in a patient with asymptomatic lung metastasis is the limiting factor in the patient's life, mastectomy may still be a logical procedure. Splenectomy for haemangiosarcoma is commonly performed but probably has little impact on survival and can be considered palliative, since it will stop the threat of immediate haemorrhage.

CONCLUSION

Surgery will be the mainstay of cancer treatment in veterinary medicine for many years to come. It is also clear that just because a surgical procedure is possible is not the best reason to do it. Although rhinotomy and curettage of the canine nasal cavity can be performed, it does not improve survival over untreated patients. Likewise, simple versus radical mastectomy in the dog does not influence survival but it may in the cat. *More surgery is not always better surgery*. Long term follow-up of well staged and graded tumours with defined surgical technique is necessary to demonstrate the true value of any operation. A great deal of progress in surgical technique and surgical thinking needs to take place before the role of surgery in cancer management can be optimized. A better understanding of expected tumour biology and more precise staging methods (angiograms, CT scans, etc) will hopefully facilitate more precise surgical operations to be performed. In spite of these anticipated advances in technology and understanding of biology, the most difficult aspect to learn is surgical judgment.

CANCERS OF THE ORAL CAVITY

Oral tumours comprise 6% of canine cancer, and 3% of feline cancer. Oral cavity is common place for neoplasia.
The most common canine oral neoplasias are:

- Malignant melanoma(MM) 30-40%
- Squamous cell carcinoma (SCC) 20-30%
- Fibrosarcomas (FSA) 10-20%
- Epulis 5-10%

In cats the most commonly seen cancer is SCC (70%) and fibrosarcomas (20%)

History:
Clinical signs at presentation include salivation, halitosis, inappetance, weight loss, exophthalmus, epistaxis, cervical lymphadenopathy (typical for tonsilar SCC, cervical lymphadenopathy can be only clinical sign). Paraneoplastic syndromes are rare, gingival haematoma was reported to be associated with hyperglycemia.

Diagnosis:

- Diagnosis is based on histopathology, melanomas can be difficult to diagnose especially in amelanotic cases and immunohistochemistry maybe required to obtain final diagnosis. Diagnosis of SCC is more straightforward, it is the most common feline oral cancer, often associated bone involvement in both species
- Unusual diagnosis of biologically high grade histologically low grade sarcoma needs to be suspected in young individuals especially Golden retrievers when fast growing oral mass with diagnosis of benign fibroma is diagnosed on biopsy.

Staging includes three views of thoracic radiographs and oral radiographs (normal radiographs do not rule out bone invasion) CT/MRI are beneficial for more accurate definition of the extent of the disease. Further staging includes biopsy, palpation of regional lymph nodes and fine needle aspirate even if normal, and 3-views thoracic radiographs.

Therapy:
Treatment modalities used for oral neoplasia include surgery, cryosurgery, radiation, chemotherapy, photodynamic therapy and combination of these.

- Surgery is most economical, fastest, most curative, complications rate reported is <15%, typically margins 2 cm are recommended and it is best determined on CT scan to plan the surgery. SCC in cat require even bigger margin and are associated with high local recurrence. Cats unlike dogs don't tolerate oral surgery well.
- Cryosurgery - lesions <2cm, minimal bone invasion, side effects oronasal fistulas, bone necrosis, fractures of bone.
- Radiation: known responsiveness as acanthomatous epulis, SCC or MM for definitive radiation
  Inoperable cancer of any histopathology for only palliation
  RT can be used to clean up post op disease (dirty margins)

Prognosis:

- Acanthomatous epulis excellent can be cured with adequate surgical resection, 90% control rate with RT, although RT is associated with 6% of bone necrosis.
- SCC is site and species dependent, canine rostral is curable with surgery or RT. Tonsils, base of tongue are highly metastatic and are associated with high local recurrence within few weeks to months after surgery. The control of feline SCC is poor, 1 year ST is <10%.
- MM is poor, 25% of patients have 1 year survival time, depends on size <2cm, (<2 cm MST 544D; >2 cm or LN MST 164D) Palliative RT an immunotherapy is reported to extend survival time.
- FSA are difficult in respect of local control, surgical margins 3-5 cm are required but not easily obtained, reported 1 year ST is 25-40%

- **Tongue neoplasia:** rare, white dogs can develop SCC, which is the most common tongue cancer (50%), followed by myoblastoma, melanoma, MCT, FSA. Feline tongue SCC is most common and is located frequently of ventral aspect of tongue and frenulum. Partial glossectomy can be performed in more than ½ of mobile tongue or ½ of longitudinal tongue. Eating and drinking can be slightly impaired, in cats grooming is compromised. Prognosis depends on location rostral better.
  Series of 10 dogs yielded 50% 1year ST with surgery and RT, 60-80% with low grade malignancy. Granular cell myoblastoma is curable with close margins- 80% permanent control rates.

- **Viral papillomatosis** horizontally transmitted by viral agent papovavirus, occurs in young animals, presents as wart like lesions, multiple in oral cavity, pharynx, tongue, lips. In majority patients spontaneous regression in 4-8 weeks.

- **Canine oral eosinophilic granuloma**: young dogs, heritable in Siberian husky, CKCS, histopathology is similar to feline EGS.Treatment with steroids and surgical resection is usually curative.

- **Epulis**; benign, gingival proliferation of tissue fibromatous or osseous, depends if ossification occurs. Firm, 1-4 cm, fixed to bone at the gum line. Treatment is conservative blade excision.

  - **Inductive fibroameloblastoma** is a rare odontogenic tumour of young cats, frequently arising from upper canine or maxilla, radiographs reveal degree of bone destruction, expansion of the maxillary bones. Teeth deformity is common. Small lesions respond to surgical debulking, large require RT. All lesions need aggressive local treatment for good local control, no metastases were reported.

  - **Nasopharyngeal polyps in cats**: young cats <2 years old, sneezing, change in voice, swallowing problems, rhinitis. Firm fleshly mass seen in caudal pharynx or above soft palate. Sometimes visualize mass in external ear canal. Skull radiographs reveal fluid or mass in tympanic bullae. Most lesions originate in Eustachian tube or bullae. Surgery is curative.

  - **Eosinophilic granuloma in cats**: "rodent ulcer", average age 5 yo, not known etiology, appear on upper lip near midline, slowly progressive lesion over years. Biopsy is needed to differentiate from other cancers. Treated with high dose of prednisone 2 mg/kg SID or methylprednisone acetate IM 20 mg/cat. Prognosis fair

CANINE LYMPHOMA

Chemotherapy is the mainstay of treatment for canine LSA. A large number of single-agent and multi-agent chemotherapy protocols have been investigated over the last 20 years. However, one optimal chemotherapy protocol has not been identified which can integrate positive outcome, toxicity and cost. In general, combination chemotherapy is considered more efficacious than single-agent chemotherapy.

Corticosteroids alone have been shown to induce at least partial remission in many dogs with LSA by their direct cytotoxic effect on the tumour cells. In addition, dogs that are systemically ill will often show improvements in appetite, activity and attitude while receiving steroids. Finally, steroids may reduce the magnitude of hypercalcaemia, if present. Oral corticosteroids (most commonly prednisone at 2 mg/kg/day initially, then tapered over time to 0.5-1 mg/kg/day) are an excellent treatment option for some owners if chemotherapy is declined. However, it is important that owners understand the ramifications of utilizing prednisone as a single agent before initiating treatment. I will commonly inform owners that "**Prednisone is a one-way street".** While most dogs will experience significant short-term improvement, the duration of that improvement is typically on the order of only 1-2 months, and *prednisone appears to be a powerful inducer of chemotherapy resistance*. In other words, multi-agent chemotherapy is much less likely to be efficacious if a patient has come out of remission after treatment with prednisone alone.

Some owners may reject the notion of utilizing injectable chemotherapeutic agents, but may be willing to combine prednisone treatment with another oral chemotherapy drug. The most common drug utilized for this purpose is oral cyclophosphamide (CTX). This combination can be administered in much the same way as it might when treating a dog with immune-mediated disease. A typical weekly dose of CTX in this setting might be 200 mg/m^2, divided into several daily oral doses. This approach may extend the median survival of LSA patients by 1 to 2 months over prednisone alone. It is important that CTX tablets not be broken or crushed, due to risks of client exposure.

A relatively simple, non-toxic and inexpensive chemotherapy protocol with intermediate efficacy is the COP (CTX/Vincristine/Prednisone) protocol. Prednisone is administered orally as above, CTX is administered either orally or injectably, and vincristine is injected weekly for several weeks, then every 3 weeks thereafter. Response rates of approximately 75% can be achieved, and the median survival times are in the range of 6-8 months in most reports. Another protocol with similar efficacy is single-agent doxorubicin (DOX). This has become more affordable for many clients since DOX has become available in a generic form, and has the advantage of requiring only one injection every three weeks. In addition, if a side effect is encountered the drug responsible is easy to identify. Two unique effects of DOX are its potential for **cumulative cardiac toxicity** and its potential to cause **severe skin necrosis if extravasated**.

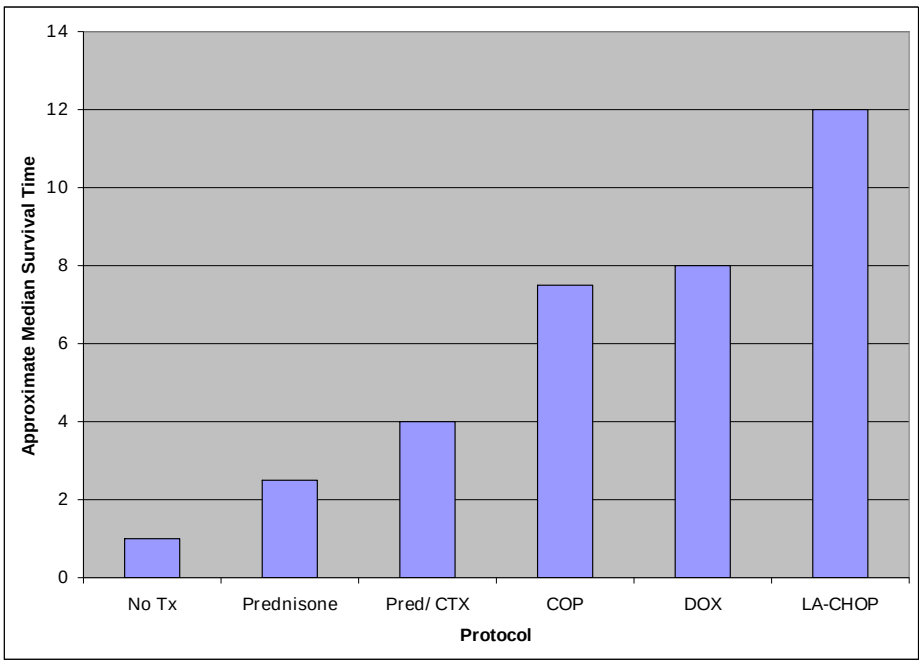
Generally, the most successful chemotherapy protocols have been *multiagent protocols that include doxorubicin*. A protocol of this type (one of many published protocols), referred to here as LA-CHOP, is employed at the UW-VMTH and at ACC. (It has also been referred to in publications as the UW-Madison protocol, or L-ASP-VCAM.) This treatment utilizes sequential injections of vincristine, CTX, and DOX, combined with daily oral prednisone for the first 4 weeks. An injection of L-asparaginase is also administered the first week.

Complete response rates are **85-90%** with these protocols, and median survival times are approximately **12 months**, with 20-25% of dogs living longer than 2 years. The likelihood of a patient experiencing some type of adverse side effect is approximately 20%. However, the vast majority of side effects observed are mild and self-limiting, and do not require hospitalization. The likelihood of a patient experiencing a severe side effect (usually refractory vomiting or diarrhea, or neutropaenia severe enough to cause sepsis) is approximately 5%. Despite the improvements made in recent years in extending disease-free interval and survival time in dogs with LSA, all but 5% of patients will eventually relapse.

Table 2: ***LA-CHOP (UW-Madison) Protocol for Canine Lymphoma***

Week 1:	Asparaginase 400 IU/kg SQ Vincristine 0.5-0.7 mg/m^2 IV Prednisone 2 mg/kg PO SID	Week 11: Vincristine 0.5-0.7 mg/m^2 IV
Week 2:	Cytoxan 200-250 mg/m^2 IV Prednisone 1.5 mg/kg PO SID	Week 13: Cytoxan 200-250 mg/m^2 IV
Week 3:	Vincristine 0.5-0.7 mg/m^2 IV Prednisone 1 mg/kg PO SID	Week 15: Vincristine 0.5-0.7 mg/m^2 IV
Week 4:	Doxorubicin 30 mg/m^2 IV Prednisone 0.5 mg/kg PO SID	Week 17: Doxorubicin 30 mg/m^2 IV
Week 6:	Vincristine 0.5-0.7 mg/m^2 IV	Week 19: Vincristine 0.5-0.7 mg/m^2 IV
Week 7:	Cytoxan 200-250 mg/m^2 IV	Week 21: Cytoxan 200-250 mg/m^2 IV
Week 8:	Vincristine 0.5-0.7 mg/m^2 IV	Week 23: Vincristine 0.5-0.7 mg/m^2 IV
Week 9:	Doxorubicin 30 mg/m^2 IV	Week 25: Doxorubicin 30 mg/m^2 IV

Continue treatments as above on an every 3 week basis (substituting mitoxantrone or actinomycin D for DOX) or discontinue and recheck regularly for recurrence.



Maintenance vs. No Maintenance

One of the current debates among veterinary oncologists centers around the utility of "extended maintenance" chemotherapy for dogs with LSA. In human medicine, treatment is rarely continued for longer than 6 to 10 months, and randomized trials have not demonstrated significant survival advantage for patients receiving extended maintenance chemotherapy. However, the dosages of chemotherapeutic agents that dogs with LSA can tolerate are less than half of what a human would receive of the same agents. Recently, we investigated the effect of discontinuing treatment after 25 weeks of standard-dose chemotherapy. Analysis of a cohort of 50 patients treated with this protocol showed *no statistical difference* in median survival time or disease-free interval when compared with

dogs receiving a similar protocol including extended maintenance chemotherapy. In addition, every dog that completed the 6-month chemotherapy protocol experienced a second complete response when the protocol was re-started at the time of relapse. More recently, we have begun to evaluate the efficacy of an "accelerated" protocol with no maintenance therapy, which entails continuing weekly therapy for a total of 4 1/2 months and then discontinuing treatment.

Rescue

When remission is lost (either after an interval with no chemotherapy or after treatment at 2 or 3 week intervals), a large number of patients may experience a second remission simply by returning to the "Top of the protocol", i.e. switching back to weekly treatments and re-initiating prednisone therapy. However, a rule of thumb is that the second remission is likely to be about half as long as the first. After a period of time, the tumour cells will acquire resistance to the initial drugs utilized, and "rescue" or "salvage" chemotherapy drugs or protocols can be considered. A summary of rescue agents/protocols that have been systematically evaluated is shown in **Table 3**.

The take-home message is that while there are many different drugs that can be utilized in this setting, no one agent or protocol is uniformly superior over the others in terms of response rate and duration. Sometimes, attaining a second or third remission can be a matter of trial and error, until an efficacious drug or protocol is found.

Table 3: ***Response to Chemotherapy in Dogs with Relapsed Lymphoma.***

Drug(s)	Resp. Rate	Resp. Duration	Comments
Actinomycin D	0-83%	0-42 days	Variable between studies
Mitoxantrone	41%	126 days	Resp. duration for CR only
VP-16 (Etoposide)	15%	8 and 90 days	Severe pruritus in 85%
CCNU (Lomustine)	24%	NR	Cum. thrombocytopaenia
Taxol	33%	NR	Hypersensitivity reactions
Doxorubicin	42%	145 days	No prior exposure to DOX
DOX/DTIC	54%	30-106 days	
Cisplatin/Cytosar	30%	30-108 days	
MOPP	88%	28 days	Severe myelosuppression
D-MAC	80%	28-86 days	12 weeks if CR attained

DTIC: Also called dacarbazine. MOPP: Mechlorethamine (nitrogen mustard), vincristine, procarbazine, prednisone. D-MAC: Dexamethasone, melphalan, actinomycin D, cytosine arabinoside. NR: Not reported. Response rates refer to a combination of complete and partial responses.

New Directions

Despite the continued development of novel chemotherapeutic agents, it is unlikely that new or additional chemotherapy drugs will have a significant impact on our ability to cure canine lymphoma. Some new approaches being evaluated for canine LSA treatment include combinations of chemotherapy with half-body radiation therapy, and the use of dose-intensified chemotherapy combined with bone marrow transplant. The UW-Madison has an ongoing clinical trial funded by the Morris Animal Foundation evaluating the merits of a combined **chemoimmunotherapy** protocol. Dogs with untreated, immunohistochemically confirmed B-cell lymphoma of WHO stages II-V, substages a or b receive a 19 week "collapsed" LA-CHOP chemotherapy protocol, followed by randomization to receive 8 weekly *autologous tumour cell vaccinations* or placebo injections.

Investigational use of recombinant immunotoxins and monoclonal antibodies has been used in people and remains to be investigated in veterinary medicine.

In summary, although canine LSA is a disease that can rarely be cured, it can be managed effectively in the majority of cases. Therapy is typically very well tolerated, and patients experience an excellent

quality of life. Significant improvements have been made in recent years with regard to the treatment of this common disease in dogs, and we are hopeful that the coming years will bring equally great improvements.

CANINE MAST CELL TUMOUR

Mast cell tumour (MCT) represents the most common malignant cutaneous tumour in the dog accounting for up to 20 % of all cutaneous canine tumours. There is a large degree of variation in the histologic appearance and biologic behavior of canine MCT, ranging from histologically and behaviorally benign to histologically and behaviorally malignant.

MCT of low or intermediate histologic grade (Patnaik Grade I or II) comprise 60 to 79% of all cutaneous MCT in the dog. These tumours exhibit quite aggressive local tissue invasion, necessitating **aggressive surgery with wide (at least 3 cm) margins.** However, their metastatic rate is relatively low (less than 10%). High-grade or undifferentiated MCT (Patnaik Grade III), in addition to being very locally infiltrative, have a considerably higher metastatic rate. Thus, aggressive surgery or other local therapies, while still necessary, are considered insufficient for optimum control. The presence of these highly metastatic undifferentiated tumours, and the necessity for major reconstructive or disfiguring surgery (e.g. amputation, body wall resection) in order to achieve histologically "clean" margins, have prompted the search for other effective treatment modalities.

Prior studies have identified several prognostic factors associated with MCT:

1) Histologic grade is one of the strongest prognostic indicators; dogs with grade III tumours typically die of their disease rapidly despite appropriate local therapy. **Median survival of dogs with grade III MCT after surgery alone is thought to be in the 3-6 month range**;

2) Clinical stage - Dogs with metastasis to regional lymph nodes or other structures at presentation having a less favorable long-term prognosis; multiple lesions don't cary worse prognosis then single lesion, always submit all masses for histopathologic evaluation as grade can vary between tumours.

3) Location - Tumours in the preputial, perianal, oral, subungual and other mucocutaneous sites typically have worse prognoses;

4) Recurrence following initial surgical excision is felt by some to be a negative prognostic indicator;
5) The presence of systemic signs (anorexia, vomiting, hematemesis, melena) is a strong negative prognostic indicator.

6) Proliferation index measured by means of AgNOR staining, PCNA, Ki-67 has been correlated with the grade, and therefore correlates with the long term outcome.

The choice of treatment modalities used for canine mast cell tumours include surgery, radiation therapy, chemotherapy and combination of these.

Careful staging by means of evaluation of regional lymph nodes, abdominal ultrasound and

In addition to aggressive local surgery, several other local therapeutic modalities have been investigated for the adjuvant treatment of canine MCT. **Radiotherapy (RT)** has proven to be a very effective local treatment modality when combined with "marginal" surgical excision. **2-year control rates of 85 to 90%** can be expected when incompletely excised *low- or intermediate-grade* MCT are treated with RT. Radiotherapy to bulky tumours is consistently less effective than RT to microscopic disease, with a one-year control rate of approximately 50%. Alternative local therapies that have been reported include hyperthermia with RT, interstitial RT, photodynamic therapy, intralesional corticosteroids, cryotherapy, and intralesional deionized water injection. None are as thoroughly investigated, clinically effective, or practical for achieving long-term local control as are appropriately aggressive surgery and/or RT.

Animals with undifferentiated MCT, MCT that have metastasized, or tumours in a historically unfavorable location (see above) may benefit from the addition of some form of systemic therapy to appropriate local therapy. In addition, aggressive surgery or RT may be declined by some owners for various reasons. Recently, several studies have been published investigating various systemic therapies for canine MCT, the results of which are summarized in **Table 4**.

Table 4: ***Response to Chemotherapy in Dogs with Mast Cell Tumours.***

Drug(s)	%CR	%PR	ORR	Median Resp. Dur.	Comments
Prednisone	4%	16%	20%	NR	
Vincristine	0%	7%	7%	NR	32% severe toxicity (?)
CCNU (Lomustine)	6%	38%	44%	79 days*	Cum. thrombocytopenia
P/C/V	0%	78%	78%	NR	
COP-HU	23%	35%	59%	53 days	

CR: Complete Response. PR: Partial (>50%) Response. ORR: Overall Response Rate. P/C/V: Prednisone/Cyclophosphamide/Vinblastine. COP-HU: Cyclophosphamide/ Vincristine/Prednisone/Hydroxyurea. NR: Not Reported

* Excludes patient that experienced CR - Euth. without evidence of disease after 440 days

The information below discusses the use of oral prednisone combined with injectable vinblastine (VBL) for the treatment of canine mast cell tumour, both in the post-surgical setting and in the setting of bulky disease.

Chemotherapy administration - Prednisone is administered orally at an initial dose of 2 mg/kg SID, and this dose was tapered and discontinued over 12 to 26 weeks. VBL was given as a rapid intravenous bolus at **2 mg/m^2**. The most common protocol consists of weekly injections for 4 weeks, followed by 4 biweekly injections. In the context of macroscopic disease, treatment is continued for as long as it is felt to be effective.

Side Effects - Adverse effects were noted in approximately 20% of patients, usually after the first dose of VBL. These were considered mild in 15%, and severe in 5%. Mild side effects include self-limiting vomiting (necessitating a 20% dose reduction), neutropaenia without evidence of sepsis (7-day neutrophil count less than 1,000/μL), and lethargy/soft stool. Severe side effects consisted of severe, refractory vomiting and febrile neutropaenia after the first VBL dose.

Response - Overall response rate in the "gross disease" population was 7/15 (**47%**), consisting of 4 complete responses and 3 partial responses, with a **median response duration of 153 days**. Survival time was significantly longer in the responding group versus the nonresponders (70 days vs. median not reached, $p = .005$). Overall median survival time in the "gross disease" group was 154 days. As an adjunctive therapy to incomplete surgical resection ("microscopic disease" group), VBL and prednisone treatment conferred a **57%** one and two-year disease free rate. Although this is less than the 85 to 90% two-year disease free rate conferred by surgery plus RT, it is our feeling that this number represents a significant improvement over incomplete resection alone. It should be pointed out that this represents a small case number, and results may vary with a larger sampling of "microscopic disease" patients.

Factors that influenced survival upon univariate analysis are listed below **(See Table 5)**. The difference in survival between the "gross disease" and other disease categories underscores the continued importance of surgery as a mainstay of treatment for canine MCT.

Table 5: ***Univariate Analysis of Prognostic Variables for Effect on Survival Time for 41 Dogs with Mast Cell Tumour Treated with Prednisone and Vinblastine***

Variable	Number of Dogs	Median Survival (Days)	*p* Value	Hazard Ratio (95% CI)
Recurrent				
Yes	10	245 d		
No	31	NR	**0.0012**	**4.372 (2.273-28.40)**
Local Treatment				
Gross	17	245 d		
Micro/ALT	24	NR	**0.0031**	**4.269 (1.697-13.64)**
AgNOR Frequency				
> 3.04	14	330 d		
≤ 3.04	13	NR	**0.0167**	**5.266 (1.303-14.18)**
Histologic Grade				
III	23	331 d		
II	18	NR	**0.0124**	**3.758 (1.318-9.756)**
Lymph Node Status				
Positive	22	355 d		
Negative	19	NR	**0.025**	**3.370 (1.155-8.302)**
Ulceration				
Yes	6	225 d		
No	35	NR	**0.033**	**3.183 (1.164-33.89)**
Prior Chemotherapy				
Yes	5	135 d		
No	36	NR	**0.0486**	**2.947 (1.010-26.21)**

Upon multivariate analysis, the overall median time to treatment failure was **317 days**, with 49% progression free at 2 years. After accounting for all other variables, statistically significant prognostic variables for treatment failure were histologic grade (***p* = 0.005)**, recurrent tumour ($p = 0.001$), and presence of gross disease ($p < 0.001$). Seven of 23 patients (30%) whose disease was controlled at least grossly by surgery ("microscopic" and "adequate local therapy" populations) developed progression of MCT. Recurrence at the site of prior tumour resection occurred in 1 patient, 5 developed disease elsewhere, and 1 had both local recurrence and distant disease. The overall MST was not reached, with a median follow-up of 579 days. 63% of patients were alive at 1 year, and 56% were alive at 2 years. Statistically significant prognostic factors for survival following multivariate analysis were recurrent tumour ($p < 0.001$) and histologic grade ($p = 0.012$).

It is interesting to note that presence of a **recurrent tumour** retained extremely strong prognostic significance for recurrence and survival after multivariate analysis. This suggests strongly that *the time to consider other types of therapy with curative intent* (i.e. aggressive re-resection, radiotherapy, or chemotherapy) *is at the time a MCT is first diagnosed, rather than at the time of recurrence.*

Prednisone and VBL provided longer survival in patients with grade III MCT than has surgery alone has in prior reports, with a MST of 331 days, and 45% of patients with grade III MCT alive at 1 and 2 years. This is an apparent improvement over historical survival data generated employing surgery alone (Various studies have quoted a median survival time of 13 weeks, a 15% 7-month survival, and a 6% 48 month survival).

CCNU (Lomustine) is a nitrosourea alkylating agent that has been used to treat canine lymphoma and brain tumours and has been shown to have activity against mast cell tumours. Reported response rate is approximately 40% in dogs treated for grade II and III mast cell tumours. CCNU has been recently used in combination with Vinblastine +/- Prednisolone. Clinical trials are currently underway to evaluate the efficacy of this combination.

Summary:

Combination chemotherapy with prednisone and vinblastine appears to be an effective therapy for canine MCT. In addition to apparently increasing the survival time of high-risk (grade III) patients after surgery, it may also be beneficial for animals with incompletely resected intermediate grade tumours where aggressive local therapy (surgery, RT) is not possible or has been declined. The cost of these drugs is relatively low, particularly in comparison to RT, and they appear to be well tolerated by the majority of canine patients.

Canine hemangiosarcoma (HSA)

HSA is a highly malignant tumour originating from endothelial cells, representing approximately 2% of all canine tumours. HSA tends to affect older dogs, with median age 10 years at diagnosis. Although any breed can be affected, German shepherds, Golden retrievers and Labradors seem predisposed.

Etiology:

Definitive etiology remains uncertain, although the strong breed association suggests a genetic predisposition. In cases of cutaneous HSA chronic ultraviolet exposure is a known risk factor in lightly pigmented breeds of dogs.

Biologic behaviour and prognosis:

As an endothelial-derived tumour, HSA can develop anywhere in the body. Spleen, heart (right atrium or auricle) skin or subcutaneous tissues and liver are the most common primary sites. Metastases occur early in disease, either hematogenously or via seeding following tumour rupture. HSA is the most common soft tissue sarcoma to metastasize to the central nervous system.
Prognosis is poor to grave except for dermal form of HSA.

Diagnosis and staging;

Due to its aggressive biologic behaviour thorough complete staging is recommended at diagnosis, in cases where emergency surgical intervention must be performed to save patients life, certain staging procedures maybe postponed temporarily.
Complete staging should include three-view thoracic radiograohs or CT scan, abdominal ultrasound, echocardiography in addition to complete blood work and urinalysis. Although UA and complete blood work rarely help to diagnose HSA, they can be suggestive of a microangiopathy (regenerative anemia, thrombocytopenia, presence of fragmented red blood cells (Schistocytes)). When suspecting HSA of the bone or CNS more advanced diagnostic imaging such as CT scan and MRI maybe required. MRI is useful diagnostic tool in planning surgery for intramuscular HSA.
Final diagnosis of HSA is based on histopathology. The 50:50 rule applies to splenic tumours. Several retrospective studies have shown that third to 50% of splenic nodules are benign and curable with splenectomy. Cytology is iin general nontraumatic and non-invasive in diagnosis in cancer in general but in cases of HSA is associated with low 25% diagnostic yield and DIC.

Therapy:

Surgery is reported to remove macroscopic disease and prevent further risk of acute haemorrhage, DIC or death. The reported survival times with surgery alone are considered purely palliative and short term, averaging between 1-3 months. Surgical resection of cutanneous (purely DERMAL ONLY) HSA is associated with median survival time of 728 days.
Chemotherapy has been reported to increase the MST to 220 days and numerous doxorubicin based protocols have been described.
Radiation therapy is used predominantly in palliative attempts (coarse fractions) for nonresectable subcutaneous or intramuscular lesions.
Novel therapy:
Immunotherapy has been a field of active research and such a rapidly progressing cancer such as HSA provides great opportunity for even small improvements to be detected. Improvement of MST to 273 days has been reported combining AC protocol with liposomal muramyl tripeptide phosphatidyletanolamine (L-MTP-PE).
Antiangiogenic therapy is an important field of investigation and systemic administration of IL12, interferon-&-2a, trombospondin and thalidomide has been investigated.
Improvement of delivery of traditional chemotherapy has been investigated and nanotechnology is one of the ways of improvement of transport as well as side effects of chemotherapy. We have been conducting clinical trials for the last 24 months for patients with hemangiosarcoma with encouraging results for histologically confirmed HSA of the heart, spleen and muscle.

Dr Martin Havlicek
Surgical Oncology

TUMOUR BIOPSY

Tissue biopsy is the key to a reasonable decision making in tumour treatment.
Properly planned and performed tissue biopsy is the prerequisite to a successful outcome in oncological surgery.
Surgical treatment could be curative option for number of tumours and biopsy should be performed in such a way as to minimize the morbidity associated with incorrect tissue sampling.

Although one-stage surgical removal of a mass has its indications in some patients, this so-called excisional biopsy should be always performed with regards to tumour type and biological behaviour.

In general, biopsy is obtained pre-treatment for proper tumour diagnosis, staging of the tumour and for reasonable planning of the best treatment option.

Basic imaging techniques, such as radiographs and ultrasound are invaluable for biopsy planning and acquisition. CT or MRI is readily available today and should implement planning of the biopsy.

Indications for biopsy

Biopsy should deliver definitive tumour diagnosis.

Biopsy should reveal a grade of the tumour. Grade gives the oncologist an indication about the biological behavior of tumour, based on cell differentiation, mitotic index and invasion of surrounding stroma.

Biopsy is indicated if the extent or type of treatment would depend on the tumour type and its grading.
Biopsy is indicated if the owner's willingness to proceed with aggressive treatment depends on the knowledge of tumour type and prognosis.

In few circumstances, pre-treatment biopsy is (in my opinion) not advisable. In the case where biopsy would be excessively invasive and dangerous and not altering the treatment option, I advise for surgical removal (excisional biopsy) followed by histopathology. (spinal cord neoplasia, brain tumour, splenic tumour (controversial) and solitary lung lobe tumour, suspected thyroid tumour)

Intra-operative histopathology is available at some academical institution but intra-operative impression smear is valuable tool where decision must be made during the operation.
The importance of histological assessment of surgical margins in excisional biopsy is extremely important in concluding the completeness of excision. Tissue ink should be used to mark the margin of concern.

Resection Margins classification according to Enneking (1983)

- **Intracapsular resection/biopsy** – debulking of tissue, leaves residual tumour in situ
- **Marginal resection** – removal just on pseudocapsule, leaves often microscopic disease
- **Wide resection** – en block resection with wide margin of healthy tissue within anatomical compartment
- **Radical resection** – margins extend beyond anatomical compartment

Biopsy technique

Proper surgical aseptic technique is mandatory.

Gentle tissue handling prevents disruption of the sample submitted. Avoid electrocautery and crushing forceps on the sample to be submitted.

Biopsy track and incision should be carried out with final surgical treatment plan in mind. All biopsy tracks should be subsequently removed during final surgical resection. Incorrectly performed biopsy can make the final surgical treatment very difficult or even impossible.

Where feasible, biopsy should avoid necrotic centre of the tumour and should involve margin between the pathology and the healthy tissue. Exception to this is primary bone cancer. Biopsy obtained from the edge of the bone lesion is often consistent with reactive bone only and needs to be repeated to achieve the full diagnosis.

Size of the biopsy matters. Large sample often provides more accurate diagnoses.
Tumour often contains area of necrosis, inflammation and reactive tissue. Pathologist should be able to get few representative sections from your sample. Tru-cut biopsy can provide multiple samples from a mass obtained via one skin stab incision.

Some skin masses can be safely sampled with Tru-cut biopsy under local anaesthesia. Avoid penetrating tissue plane or anatomical compartment with the biopsy needle.

For larger excisional biopsies, consider skin tension lines when planning the final surgical procedure.

Impression smear can be obtained at the time of biopsy.

Always submit your excisional biopsy to the pathologist. ("What is worth of removing, is worth of identifying" – S.Withrow)

Type of biopsy - FNAB,
- punch biopsy
- needle core or Tru-cut biopsy
- incisional biopsy
- excisional biopsy
- laparoscopic/thoracoscopic assisted biopsy
- endoscopic biopsy

FNAB - Cytology vs histology

Cytology is evaluation of cells harvested from lesion by scrapes, smears or by fine needle aspiration biopsy (FNAB)

Fine needle aspirate biopsy in veterinary medicine is well established rapid screening test. It produces reliable diagnosis of some tumours (e.g. round cell tumour, some spindle cell tumours) and most-importantly, distinguishes tumour from non-neoplastic lesions.

FNAB safely identifies e.g. mast cell tumour, lymphoma, histiocytoma or plasmacytoma and is useful for surgical planning but will not disclose grade of the tumour and should optimally be followed by histological evaluation.

FNAB has high diagnostic yield. It is simple and produces rapid result non-invasive way with low morbidity. FNAB of cutaneous and subcutaneous lesions has a sensitivity of 89.3% and specificity of 97.9%. The tumour cell type (epithelial, mesenchymal, round, melanoma) were correctly determined in all positive cytologic diagnoses by Ghisleni et al (2006).

FNAB of the lung - may be complicated by pneumothorax, haemorrhage, infection and air emboli. Tumour cell implantation during lung FNAB in human is very rare, occurring in 1/1264 cases (0.08%) as reported by Sinner (1976); in 0/1500 (0%) reported by Lalli et al (1978) and in 2/66 (3%) reported by Harrison et al (1984).

Some authors suggest that FNAB of the lung should not be performed to lesion likely to be malignant and operable and advocate open biopsy excision based on CT signs of malignancy (Wollinsky,1969). In contrast, one extensive study involving 8.607 human patients with stage 1 lung cancer concluded that percutaneous transthoracic needle biopsy was not associated an increased risk of death (Wisnievesky et al, 2006) and poorer survival.

FNAB of appendicular osteosarcoma – according to Britt et all (2007), diagnostic sample was obtained in 32 of the 36 cases. Of the 32 cases, cytology indicated sarcoma, with a sensitivity of 97% and a specificity of 100%

When using fine (23G) needle, aspirates of abdominal organs are generally well tolerated by dogs under light sedation. FNA of liver, spleen and intra-abdominal lymph nodes is very important part of oncological staging process.

Tissue to biopsy

Skin and subcutaneous masses – punch biopsy, incisional or excisional biopsy, Tru-cut or needle core biopsy are all appropriate technique for dermal and subdermal lesions. Size of the sample is usually guided by the mass location. For excisional biopsy, use tissue ink to mark your margin of concern prior submission.

Bone biopsy – Jamshidi bone biopsy needle or Michelle trephine are the most common technique. Although Michelle trephine provides larger sample, it is more likely to cause iatrogenic bone fracture. Jamshidi needle, when guided towards the lesion via stab incision, yields satisfactory tissue sample without surgical approach. Sample bone from the centre of the lesion. Jamshidi needle biopsy has an accuracy rate of 91.9% for detecting tumour versus other disorders and an 82.3% accuracy rate for diagnosis of specific tumour subtype.
Fine needle aspirate with 91G needle may reveal spindle cells in 40-60% of cases and may help to rule out fungal or bacterial osteomyelitis, where inflammatory cells or organisms may be observed.

Lymph node – FNA and needle core biopsy are used by internists while node extirpation is preferred by a surgeon.

Oral lesion – incisional wedge biopsy is the preferred option. It is often nearly impossible to localize the extent of oral mass when excisional biopsy was performed by surgeon other then the one performing the final, curative intent resection. CT should be always used to assess bone involvement.

Nasal lesion- good quality radiographs or preferably CT scan is very useful for evaluating the extent of any mass within the nasal passage and nasal sinuses. Rigid rhinoscope allows for visualization of rostrally located lesions. Alligator or clam biopsy forceps under direct visual guidance is used for sample collection. Flexible scope with biopsy channel can be used to biopsy lesion rostrally or retroflexed via oropharynx caudally. In medium-to-large dog, a technique using plastic rigid catheter sleeve attached to a needle, serrated with a scalpel blade produces large tissue sample. It is crucial during this procedure to prevent inadvertent perforation of the cribriform plate by measuring the length from the nasal planum to medial canthus of the eye. Haemorrhage is encountered but is usually self-limiting.

Toe/nail – onychectomy or distal phalanx amputation is my preferred option for ungual/ subungual pathology.

Abdominal biopsy – hepatic, splenic, prostatic, intestinal lesion etc, can be aspirated with fine needle or biopsied with Tru-cut needle under direct ultrasound visualization. Percutaneous liver biopsy with core needle is safe but should be preceeded by laboratory coagulation profile.
Advances in endoscopy and laparoscopy greatly enhanced our ability to achieve diagnosis without invasive surgery.
Flexible endoscopy is used for assessment of hollow viscus such as upper or lower GI tract or urinary bladder. Endoscope with a biopsy channel facilitates directly visualized lesion sampling of mucosal surface. Laparoscopy on the other hand is an excellent option for assessment of the visceral surface of the coelomic cavity. It should be used for staging, surgical planning, assessment of feasibility of surgical resection and for biopsy prior definitive surgery.

Exploratory laparotomy allows for direct visualization of the pathology and enables definitive excision or at least incisional biopsy with regional lymph node evaluation.

Neck lesion – location of the lesion on the neck is usually sufficient to make a tentative diagnosis. Salivary gland tumour should be satisfactorily diagnosed from sialocoele, foreign body or lymphadenomegaly by fine needle aspirate. Should this not provide the diagnosis, incisional biopsy can be safely performed.
When thyroid tumour is suspected, ultrasonography or contrast CT is very useful for evaluating the invasion of adjacent structures as well as metastasis. Chest radiographs or CT should be mandatory. Patient with thyroid lesion which is mobile and without distant metastasis is considered to be surgical candidate and excisional biopsy is recommended. Incisional biopsy or FNA should be avoid due to high risk of profound haemorrhage and tumour spread. The thyroid gland should be always approached via ventral midline. Improperly driven biopsy warrants unnecessarily extensive and dangerous resection involving all adjacent contaminated structures. Ventral midline approach allows for through search for metastasis along the neck.

Thoracic cavity - Lung tumours primary and metastatic
- Tumours of the chest wall
- Thymic and lymphatic tumours
- Tumours of the heart
- Mesothelioma

Biopsy of thoracic mass is in my opinion indicated only for lesions involving thoracic wall and for differentiation of thymoma vs lymphosarcoma. Successful surgical resection of thymoma can produce long survival times while lymphosarcoma is considered medical condition and surgical removal is of low benefit. Cranial mediastinal mass or lymph node can be aspirated under direct ultrasound guidance with FNA or core needle.
I consider thoracoscopy the procedure of choice for thoracic exploration and biopsy of pleural lining. Successful thoracospic lung lobectomy has been already reported for canine primary lung masses.

Majority of oncology surgeons conclude that FNA or biopsy of primary lung tumours is unnecessary and the result will not alter the ultimate treatment.

TUMOURS OF THE SKELETAL SYSTEM

Osteosarcoma (OS) is the most common primary bone tumour in dogs accounting for up to 85% of malignancies originating in the skeleton (Dernell, 2003). Primary OSA represents 5% of all canine tumours. Chondrosarcoma is the second most common primary tumour of the bone in humans and dogs and accounts for approximately 5-10% of all canine primary bone tumours. CSA most commonly occurs in flat bones and tumour location appears more prognostic then histological grade (Sylvestre et al., 1992). Primary haemangiosarcoma of bone is rare (<5% of all bone tumours). Prognosis for bone HAS is poor, with <10% probability of surviving 1 year despite the treatment.
Multilobular osteochondrosarcoma is an unusual tumour generally arising form the skull of a dog. Local MLO excision appears to have a good long term control with histologically confirmed complete excision.

Incidence and signalment

Osteosarcoma is predominantly a disease of large, middle-aged to older dogs with a median age of 7 years and a small peak incidence in 1-2 years (Misdorp and Hart, 1979).
In a study of Kistler, which reviewed 1462 cases of canine OS, dogs weighing more than 40 kg accounted for 29% of all cases and only 5% of their tumours occurred in the axial skeleton. Only 5% of OSA's occur in dogs weighing less than 15 kg but 59% of their tumours originated in the axial skeleton.
The breeds most at risk are Rottweilers, Great Danes, Irish Woolfhounds, St Bernard, German Shepherd and Golden Retrievers (McNeill et al., 2007).

Risk Factors for the development of osteosarcoma

The etiology of canine OS is generally unknown.
Bone infarcts are rarely implicated in the development of canine OS, and the pathogenesis of OS in dogs with bone infarcts remains unknown.
There are reports of OSA associated with metallic implants used for fracture repair and in cases of fractures without internal fixation.
Ionizing radiation, in both experimental and therapeutic settings, has been known to cause osteosarcoma. Three of 87 spontaneous tumour-bearing dogs or 3.4% of dogs treated for soft tissue sarcomas developed OS within the field of radiation between 1.7 to 5 years after radiation (RT).
Any chronic inflammatory process may induce bone or soft tissue sarcoma.
Fatigue microdamage of the methaphyses has been proposed as a potential risk factor for OS in large breed dogs. A theory based on circumstantial evidence is that, since OS tends to occur in major weight bearing bones adjacent to late closing physes, and heavy dogs are predisposed, multiple minor trauma and subsequent injury to sensitive cells in the physeal region may occur.
Alterations in the tumour-suppressor genes Rb and p53 are commonly identified in human patients with osteosarcoma and have been studied in canine patients (Lokopoulos et al. 2003; Kirpensteijn, 2007).

Pathogenesis

Osteosarcoma is a malignant mesenchymal tumour of primitive bone cells. These cells produce an extracellular matrix of osteoid and the presence of tumour osteoid is the basis for the histological diagnosis differentiating OS from other sarcomas of bone. There are many histological sub classifications of OS based on the type and amount of matrix and characteristics of the cells: osteoblastic, chondroblastic, fibroblastic, poorly differentiated and telangiectatic osteosarcoma.

Osteosarcoma has very aggressive local effects and causes lysis and/or production of bone (Dernell et al., 2001). The local disease is usually accompanied by soft tissue swelling. Pathological fracture of the affected bone may occur. Metastasis is very common and arises early in the course of the disease, although less than 5% of dogs have radiographically detectable pulmonary metastasis at presentation.

Approximately 90% of patient will have occult disease at the time of presentation and will die to metastatic disease. Metastasis via the haematogenous route is most common, however in 6% of cases extension to regional lymph nodes may occur. Lung is the most commonly reported site for metastasis, tumour spread to bones or other soft tissue sites can occur.

Approximately 75% of OSA's occur in the appendicular skeleton with the metaphyseal region of long bones most commonly affected. The distal radius and proximal humerus are the two most common locations.

Survival of dogs with OS distal to the antebrachiocarpal or tarsocrural joints was somewhat longer (median of 466 days) than survival of dogs with OS of more common appendicular sites however OS in these sites is aggressive with a high potential for metastasis.

Osteosarcoma usually originates within the medullary canal of bones (intraosseous-OS) however there are forms of this cancer that originate from the outside surface of bones. Periosteal OS is a high-grade form of surface OS and seems to arise from the periosteal surface but has invasive characteristics seen radiographically. There is cortical lysis with extension of the tumour into the bone and surrounding soft tissues. These tumours are histologically similar to intraosseous OS and have similar aggressive biological behavior.

Parosteal OS, or juxtacortical OS, arises from the periosteal surface of bones but appear less aggressive than periosteal OS both radiographically and in terms of biological behavior. Parosteal osteosarcomas have a moderately well circumscribed radiographic appearance. The tumours grow out from the periosteal side of a cortex and cortical lysis is usually very mild if apparent at all on radiographs. Parosteal OS is usually slow growing but can induce pain at the local site. Metastases can occur but the prognosis for survival is much better than for intra-osseous osteosarcoma. Control of parosteal OS can be achieved by en bloc resection of the tumour with the adjacent cortical bone.

Prognostic factors

Elevated alkaline phosphatase (ALP) levels (total, liver or bone isoenzymes) have been recognized as negative prognostic factor in humans and dogs (Ehrhart, 1998). Dogs with elevated ALP levels have shorter survival times (ST) by approximately 50%.

Breed and sex does not appear to have prognostic significance but young dogs with OSA appear to have shorter ST and biologically more aggressive disease.

Percent tumour necrosis, induced by RT or chemotherapy prior tumour excision is predictive of local tumour control.

Multiple studies recognized presence of detectable metastatic disease at the time of diagnosis poor prognostic factor, and standard chemotherapy being ineffective in improving survival in these cases.

Diagnosis and work up

Presumptive diagnosis of OSA can be made based on signalment, history, physical examination and radiography.

Differential diagnosis of lytic, proliferative or mixed bone pattern identified on radiographs include other primary bone tumours, metastatic bone tumours, multiple myeloma or lymphoma, haemangiosarcoma, systemic mycosis (disseminated aspergillosis caused by *Aspergillus terreus*) and bacterial osteomyelitis.

Recommended minimum database consists of CBC, serum biochemistry and urinalysis to screen for concurrent disease and help to establish prognosis by quantitating the serum ALP concentration.

Further staging of patient with osteosarcoma includes diagnostic imaging. Initial evaluation of the primary site involves interpretation of radiographs taken in lateral and craniocaudal projections. Cortical lysis is a common feature of OS and may be severe enough to leave obvious areas of discontinuity of the cortex leading to pathological fracture. There is often soft tissue extension with an

obvious soft tissue swelling and new bone (tumour bone) may form in these areas in a palisading pattern perpendicular or radiating from the axis of the cortex ("sun-burst").

There is often loss of the fine trabecular pattern in the metaphysis, a vague transition zone at the periphery of the medullary extent of the lesion or areas of fine punctate lysis.

Osteosarcoma does not directly cross articular cartilage.

Oncological staging and examination for evidence of apparent spread of the disease is important. Regional lymph nodes should be palpated and fine needle cytology performed on any enlarged node.

Thoracic radiographs reveal gross metastatic disease in less than 10% of dogs at the time of presentation. Thoracic radiographs should be taken during inspiration with the patient awake (sedated), and should include three views: a ventrodorsal view or a dorsoventral view and both right and left lateral views.

Sites of bone metastasis may be detected by a careful orthopedic examination with palpation of long bones and the accessible axial skeleton. Bone survey radiography has been useful in detecting dogs with second skeletal sites of osteosarcoma. Reported incidence of multiple lesions is 4-7%.

Computerized tomography may be useful to plan surgery especially for tumours located in the axial skeleton. CT proved to be most accurate at predicting tumour length when compared to MRI and traditional radiography (Davis et al., 2002). Magnetic resonance imaging can also be used to stage local disease. This is a valuable tool to determine the extent of the soft tissue component of the tumour especially within the medullary canal and in the soft tissue outside the cortex.

Nuclear scintigraphy significantly overestimates tumour length when used for surgical planning (Leibman et al., 2001)

Although biopsy remains the gold standard for diagnosis of OS, fine needle aspirate via 19-gauge needle may provide diagnosis of sarcoma in 40-60% of cases by less invasive means.

A surgical staging system for sarcomas of the skeleton has been devised for people. This system is based on the histological grade (G), the anatomic setting of the primary tumour (T) and regional or distant metastasis (M). There are three stages: stage I, the low-grade (G_1) lesions with out metastasis; stage II, the high-grade (G_2) lesions without metastasis; and stage III, the lesion with regional or distant metastasis regardless of histological grade. The stages are subdivided by the anatomic setting, A being intracompartmental (T_1) and B extracompartmental (T_2). According to this system, most dogs with OS present with stage IIB disease.

Treatment

Standard of care providing consistently the longest survival is surgical resection followed by adjuvant chemotherapy. Surgery alone, in dogs with no evidence of metastatic disease, is associated with median survival time of 19 weeks. With no treatment at all, dogs become very painful because of extensive destruction of bone and surrounding tissue by their primary tumours and most owners elect euthanasia for their pets soon after diagnosis if no treatment is given. Surgery alone is appropriately considered palliative for osteosarcoma.

Surgery

Surgical options for appendicular OS include amputation and limb preserving procedure. Amputation of the affected limb is the standard treatment for canine appendicular osteosarcoma. Even large and giant breed dogs can function well after limb amputation and 95% of owners are pleased with their pets' mobility and quality of life after surgery. It is important to rule out preexisting orthopedic or neurological conditions, which may cause poor results in some cases

Although most dogs function well with amputation, there are some dogs where limbsparing would be preferred over amputation, such as dogs with preexisting orthopedic or neurological disease, very large dogs, or dogs with owners who absolutely will not permit amputation.

Limbsparing surgery involving resection of the affected bone and replacement of that by bone allograft (metaphyseal or intercalary) with or without polymethyl metacrylate reinforcement (Kirpensteijn et al.,1998) is probably the most commonly performed technique.

The resected diseased bone can be pasteurized, autoclaved or irradiated and returned to the donor site (Liptak et al.,2004)

Limbsparing technique utilizing free vascularized autograft or ipsilateral vascularized ulnar transport autograft has been reported (Seguin, 2003).

Bone graft seems to be successfully replaced by stainless steel endoprosthesis (Veterinary Orthopaedic Implants, Veterinary Instrumentation) with superior results and lower infection rate.

Ehrhart (2005) reported distraction osteogenesis technique with circular external fixateur for a successful limbsparing procedure.

The most suitable candidates for limbsparing are dogs with tumours in the distal radius or ulna. Criteria for determining a patient's eligibility for limbsparing surgery include the primary tumour affecting < 50% of the bone determined radiographically and tumour not extending across the joint, no evidence of metastatic and concurrent disease.

Adjuvant treatment

Chemotherapy protocol using platinum based drugs or doxorubicin or combination of these drugs is widely used for treatment of canine OS. Cisplatin used either alone or in combination with doxorubicin on an alternating basis has been demonstrated to improve survival in dogs with OS after amputation.

The recommended dose for cisplatin is 70 mg/m2 body surface area. Saline diuresis helps prevent nephrotoxicity, which is the dose-limiting toxicity in dogs. The protocol recommended is that described by Ogilvie, et al.

Carboplatin is a second generation platinum compound that is less nephrotoxic than cisplatin with apparently similar anti-tumour effects. In a multi-institutional study of 48 dogs with appendicular OS treated with amputation and up to four doses of carboplatin the median disease-free interval was 257 days, the median survival was 321 days, and 35.4% of dogs were alive at one year. The drug can be given at amputation and every 21 days, provided there are no signs of severe bone marrow suppression. The dose recommended for use in dogs is 300 mg/m administered every 3 weeks for four treatments however the maximum tolerated cumulative dose has not been described.

A poor response to adjuvant doxorubicin as a single agent was determined by one report of 16 dogs with osteosarcoma. In that study doxorubicin was given intravenously at a dosage of 30 mg/m2 every 3 weeks, beginning 3 weeks after surgery. In a more recent study, doxorubicin was given at the same dosage but every two weeks for five treatments to 35 dogs with appendicular OS and surgical excision was performed either 13 days after the second or third treatment with the subsequent treatment given on the day after surgery. The 1- and 2-year survival rates were 50.5 and 9.7% respectively. Why the 2-year survival is so poor is unclear.

There have been several reports of adjuvant chemotherapy protocols for dogs with osteosarcoma. It would seem reasonable that combinations of cisplatin, carboplatin and doxorubicin, drugs shown to be efficacious alone, could further improve survival times.

Palliative therapy

Palliative therapy can be employed in stage III patients (metastatic disease), in patients with concurrent disease where amputation or adjuvant chemotherapy is contraindicated, or where the owner does not want to pursue any other treatment modalities.

Reported survival time for dogs treated with palliative treatment range from 3-10 months. Surgical amputation alone or along with administration of NSAIDs or opiods may significantly improve quality of life. The median survival time reported being 19 weeks.

Recent studies suggest that bisphosphonates (BP) are antiresorptive agents and act mainly by inducing osteoclast apoptosis (cell death). The newer and more potent BPs (pamidronate, zoledronate) have been used in treatment of human osteoporosis, Paget's disease, hypercalcemia of malignancy and management of metastatic bone lytic lesions secondary to prostatic and breast cancer.

The nonsteroidal anti-inflamatory drug, piroxicam may be given at 0.3 mg/kg SID. This appears to give temporary pain relief to most dogs with OS lesions. Gastrointestinal toxicity appears to be potential complication. It is also not wise to give piroxicam concurrently with cisplatin or in dogs with decreased renal function.

Some studies are suggesting that feldene may have some anti-tumour effects

Radiation

Palliative radiation for primary OS has been described. This appears to be a viable treatment option for dogs with stage III disease at presentation (metastasis to lung) or where amputation is contraindicated.

Palliative radiation typically involves administering coarse fractions of 8-10 Gy of megavoltage radiation in 3 treatments at 0,7, and 21 days. Palliative RT reportedly achieves pain relief and limb function in 75% of patients for median of 2 months duration. Recently, Green reported that palliative RT delivered at 4x8gy on days 0,7,14, and 21 caused response in 92% of patients and the median survival time was 313 days.

The use of Samarium has been described for appendicular and axial OS in dogs (Barnard et al., 2007) Samarium-153-ethylenediamine-tetramethylene-phosphonic acid (Sm-EDTMP) is a radiopharmaceutical, which emits ß particles providing therapeutic effect.

Early results with stereotactic radiosurgery (SRS) were recently reported by Farese et al. (2004). SRS is a treatment modality where the entire radiation dose is delivered in single treatment via multiple, noncoplanar beams stereotactically focused on the bone lesion. SRS minimizes damage to surrounding tissues by relying on extreme accuracy of radiation delivered to a tumour and a steep dose gradient between the tumour and surrounding healthy tissues.

In the above mentioned report, 5 dogs were treated with SRS alone and 6 dogs with SRS+Chx. 4 of the 11 dogs developed pathological fracture with mean time to fracture of 5.8 months. Median survival time achieved was 363days (145-763d) and limb function was reported good to excellent in all dogs for the first 3months after the treatment.

Metastatic Disease

The most common cause of death in humans and dogs following amputation as the sole treatment for osteosarcoma is diffuse pulmonary metastasis. Resection of pulmonary metastasis from osteosarcoma or other solid tumours has been reported in people. There is a report of 36 dogs treated with pulmonary metastasectomy for osteosarcoma (O'Brien et al, 1993). Although the initial treatments varied between dogs, the median survival time of the entire group was 487 days. The median survival after pulmonary metastasectomy was 176 days (range 20 to 1495 days). The criteria established for case selection for pulmonary metastasectomy in order to maximize the probability long survival periods are: 1) primary tumour in complete remission, preferably for a long relapse-free interval (> 300 days); 2) one or two nodules visible on plain thoracic radiographs; 4) cancer only found in the lung (negative bone scan); and perhaps 3) long doubling time (> 30 days) with no new visible lesions within this time.

Analgesics used for chronic cancer pain in cats according to Lascelles (2003)

Drug	Feline dose	Comments
Amitriptyline	0.5-2.0 mg/kg orally sid	Appears to be well tolerated for up to 12 months daily. Useful addition to NSAIDs.
Aspirin	10 mg/kg orally eod	Can cause significant GI ulceration
Buprenorphine	0.02 mg/kg sub-lingual every 6-7 hours	Predictable analgesia for 6 hours. Same dose IV provides analgesia for same period. Well received by cats. Some owners claim anorexia after 2-3 days. Smaller doses (5-10 μg/kg) more appropriate for long term.
Butorphanol	0.2-1.0 mg/kg orally qid	Generally considered a poor analgesic for cats except for visceral pain. Useful as a part of multimodal approach.
Ketoprofen	1 mg/kg orally sid for a max 5 days	Probably well tolerated as pulse therapy for chronic pain, with a few days 'rest' between treatments. Used by some at 1mg/kg every 3 days long term
Meloxicam	0.2 mg/kg orally on Day 1, then 0.1 mg/kg orally sid for 4 days, then 0.05 mg/kg sid for 10 days, then 0.025mg/kg sid	Particularly well received orally due to formulation. Easy dosage by drops.
Morphine	0.2-0.5 mg/kg orally tid-qid	May not be as effective as in dogs. Some cats strongly resent it
Paracetamol	**CONTRAINDICATED**	**SMALL DOSES RAPIDLY CAUSE DEATH**
Piroxicam	Up to 1mg/cat orally sid for up to 7 days. For long term, consider EOD	Lascelles claims that significant drop in PCV (presumably due GI haemorrhage) occurs in up to 30% of cats after 2-3 weeks therapy. Alternate- day dosing suggested for long term treatment
Tramadol	4mg/kg bid	Not evaluated for toxicity yet, but early results encouraging.
Fentanyl (transdermal)	2-5 μg/kg/h	Patch can be applied to an average cat (3.5-5kg). Do not cut in half. Half covered patch gives unpredictable results.

Analgesics useful for the treatment of chronic cancer pain in the dog as reported by Lascelles (2003)

Drug	Canine dose	Comments
Amitriptyline	0.5-2.0 mg/kg orally sid	Useful for neuropathic pain esp. when combined with NSAID. Weak analgesic. No clinical toxicity data available
Aspirin	10 mg/kg orally bid	Significantly higher risk of GI ulceration than approved NSAIDs. Inhibits platelets function
Butorphanol	0.2-0.5 mg/kg orally up to tid	Causes sedation and not very predictable analgesic. Best used in combination with NSAIDs
Codeine	0.5-2.0 mg/kg orally sid	Sedation at higher doses. Constipation.
Carprofen	2.0 mg/kg bid or 4.0 mg/kg sid	COX-1 sparing NSAID. Moderately preferential COX-2 inhibitor.
Deracoxib	3-4 mg/kg orally sid for 7 days then 1-2 mg/kg orally sid long term	Most specific COX-2 inhibitor approved for use in dogs (USA). Not to be used in dogs with existing GI ulcer as may inhibit healing.
Fentanyl (transdermal)	2-5 µg/kg/h	Very useful in short-term pain control. Limited use for long-term treatment. Need to change every 4-5 days. Expensive.
Gabapentin	3-10 mg/kg orally sid-bid	Used as anti-seizure drug. Could be useful for neuropathic pain alone or as a part of multimodal treatment.
Meloxicam	0.2 mg/kg orally on day 1. then 0.1 mg/kg sid	Preferential COX-2 inhibitor.
Morphine	0.2-0.5 mg/kg liquid form orally tid–qid 0.5–3 mg/kg sustained release orally tid-qid	Sedation and constipation. Doses >0.5-1mg/kg often cause unacceptable constipation according to some owners.
Pamidronate	1–1.5 mg/kg, diluted in 250ml of saline slow IV, probably once a month	Bisphosphonate inhibiting osteoclast activity. Useful in primary or metastatic bone pathology causing osteolysis.
Paracetamol (acetaminophen)	10-15 mg/kg orally tid for 5 days. Long term up to 10 mg/kg bid	Apparently fewer GI side effects than other NSAIDs. No renal toxicity noted. Can be combined with other NSAIDs (e.g. meloxicam or carprofen)
Piroxicam	0.3 mg/kg orally sid or eod	Used in some chemotherapy protocols for its anti-angiogenic, immuno-modulatory and anti-inflammatory potential.
Tramadol	2-4 mg/kg orally bid-qid	Useful alone or as adjunctive analgesic with NSAIDs

TUMOURS OF THE THORAX

Tumours of the thoracic cavity can be broadly categorized as lesions of the thoracic wall, pulmonary parenchyma, or mediastinum with its organs (including heart, trachea, oesophagus, lymphatic tissue and thymus).
Each category represents different clinical signs, different staging process, different treatment and ultimately different prognosis.

Lung cancer

Primary lung tumours are rare, accounting for 1% of all canine and 0.5% of all feline neoplasia. Far more common in small animal practice is metastatic lung cancer. Median age at presentation is 10 years with no sex predilection.
Etiology – urban living and passive smoking has been questioned.
The most common type in both spp is adenocarcinoma, classified as differentiated or undifferentiated and according to location – bronchial, bronchoalveolar or alveolar.
Primary lung tumour metastasize to CNS in 50% of canine undifferentiated ACA and in 75% of cats.
Other forms of lung tumours such as Squamous cell carcinoma and anaplastic carcinoma of large of small cell are less common.
Differential diagnoses for solitary lung lesion includes pulmonary neoplasia, mediastinal neoplasia, pulmonary abscess, pulmonary granuloma, lung lobe torsion or focal atelectasis.
Clinical signs are related to the size of the lesion (> 2cm in dog or 0.5 cm in cat) - cough nonproductive, exercise intolerance, occasionally hemo/pneumothorax, pleural effusion are frequently observed. The presence of lameness and swelling of extremities could indicate hypertrophic osteopathy (Marie's disease).
Thoracic radiographs often reveal well demarcated mass visible within the parenchyma on all thoracic views (both lateral and V-D). Caudal lobe appears most commonly affected. MRI and CT allow more accurate assessment for resectability and occult metastases.
Bronchoscopy may be of value in centrally positioned tumours. BAL is often unrewarding except for diffuse LSA.
Treatment – surgical resection, 4.-6.th intercostal space thoracotomy allows for complete lung removal with adjacent hilar lymph node extirpation. Midline approach is better for both lung inspection and large tumour resection, but limits lymph node evaluation.
Complete lobectomy is relatively quickly performed with thoraco-abdominal stapler and is very well tolerated.
Radiation treatment is limited because normal lung tissue does not tolerate doses required, leading to fibrosis.
Prognosis is size and lymph node involvement dependent. For tumours <5 cm without clinical signs and metastases, the survival times extend over 12 months. Adenocarcinoma has better prognosis (MST of 19m) than SCC (MST 8m) according to Mehlaff et al., 1983.
Dogs with no clinical signs had a median survival of 18 months and those with clinical signs had a median survival of 8 months (McNeil et al., 1997)
Dogs in which the hillar lymph node tested positive survived median of 1 months.
Metastatic lung cancer is rarely treatable. Published criteria for surgery require >300 days in complete remission with max of 3 nodules, no other site and more then 40days doubling time. 20-25% of patients survive 12 months (O'Brien et al., 1993).

Thoracic wall

Masses of the thoracic wall most commonly involve ribs, less frequently sternum. They are rare in the dog and even rarer in the cat. Bone tumours most commonly occur at the costo-chondral junction and large breed dogs are over-represented in the study of Pirkey-Ehrhart et al..(1995).
The most common neoplasms of the thoracic wall are of mesenchymal origin – osteosarcoma, followed by chondrosarcoma and soft tissue sarcoma.
Most common clinical sign is a visible mass of the chest wall, sometime accompanied by weight loss, lethargy, dyspnoea and lameness.
Appropriate imaging is crucial. Good quality, at least two views thoracic radiographs should be obtained. CT should provide information about the extent into the thoracic cavity and lung metastases. Often the lesion is larger on the inner, pleural aspect creating so called 'iceberg effect'.

CT evaluation is invaluable for surgical planning.
All thoracic wall masses should be biopsied prior to treatment!
Definitive treatment for chest wall mass is surgical en bloc resection with wide surgical margin. Ideally, at least one normal rib cranially and caudally to the mass should be removed. At least 3 cm margin of grossly normal tissue should be removed with the tumour.
Removal of more then 3 ribs present a surgeon with a challenging wound closure. Large defects often require some sort of reconstruction technique, utilizing local transposition of myocutaneous flap (e.g. latissimus dorsi or diaphragm) or synthetic mesh implantation. Surrounding soft tissue is often sufficient for defect involving less then 7 ribs but the closure must be rigid to prevent paradoxical respiration (Kuntz, 1998).

Anaesthetic protocol require intermittent positive pressure ventilation. Regional intercostals block is very useful adjunct to systemic analgesia.

Prognosis for thoracic wall masses depends on the histological diagnosis and completeness of the excision. The MST published for chondrosarcoma is 1080 days, for osteosarcoma with chemotherapy 240 days (Pirkey-Ehrhart et al., 1995)
Generally, the biological behaviour of rib osteosarcoma is similar to its appendicular counterpart.

Tumours of the mediastinal space

Differential diagnoses for most mediastinal tumours include branchial cyst, ectopic thyroid tumours, chemodectoma, thymoma and lymphosarcoma. Oesophageal, tracheal and cardiac tumours occur very rarely in the dog and cat. Mesothelioma and lymphosarcoma is considered a medical condition.

Thymoma

The thymus arises from the 3rd and 4th pharyngeal pouches. It is largest in size around 5 months of age, and then atrophies, but never disappears completely. The thymus is responsible for CMI (T cells). Not surprisingly an association exists between thymoma and certain disorders of immunity. Concurrent other non thymic cancers are common.

Thymoma most often occurs in middle-aged dogs with no sex predilection. Clinical signs include coughing, lethargy, weakness, dyspnoea, anorexia and vomiting or regurgitation. In one study, paraneoplastic syndromes were present in 67% of dogs with thymoma (Atwater, 1994). Myasthenia gravis was found in 47% of patients (Ahronson, 1984)
FNA or needle core biopsy is usually performed under an ultrasound guidance and it is critical for surgical planning. Lymphosarcoma is considered medical condition and thoracotomy is not indicated in such a case.

Not usually metastatic although has been reported. Metastatic sites reported include: lungs, liver, spleen, diaphragm, bronchial and mediastinal lymph nodes, pericardium, kidneys, and bone.

Surgical resection via midline sternotomy is the treatment of choice for thymoma. Neoplastic component is epithelium. Thymomas can be well encapsulated and noninvasive, so-called benign thymomas, or poorly encapsulated and locally invasive into adjacent structures.
Approximately 50% of thymomas are resectable, regardless of their size or CT/MRI appearance.

Prognosis for thymoma depends on its resectability and presence of myasthenia gravis.

Dogs without megaoesophagus and completely resected thymoma have been reported to have an 83% one year survival.

Myasthenia gravis may or may not resolve after successful surgery and aspiration pneumonia must be discussed with the client.

Post-operative care of thoracotomy patient

A thoracostomy tube will be required post-operatively to facilitate air and fluid drainage.
Patient with chest drain should not be ever left unsupervised!
It is useful to cover the chest drain with a thoracic bandage, to prevent self mutilation and accidental damage to the chest tube leading to fatal pneumothorax .

Thoracic drain should be postoperatively removed as soon as it becomes negative or when production of fluid decreases to less then 8mL/kg/24h.

Systemic analgesia, combined with regional intercostals block can be supplemented by local analgesic instillation in the chest cavity via the chest drain.

Dr David Collins
Internal Medicine

ANAEMIA – IS IT REGENERATING? WHY NOT?

Anaemia is defined as a reduction in the number of circulating red blood cells, haematocrit and haemoglobin, resulting in decrease in oxygen carrying capacity.

Grading the severity may be helpful in determining the aetiology. Mild anaemias (30-37% dogs; 20-26% cats) are often secondary to other disease conditions, such as anaemia of inflammatory disease, neoplasia, hepatic, renal or endocrine disease.

Moderate anaemias: 20-29% dogs; 14-19% cats.
Severe anaemias: 13-19% dogs; 10-13% cats.

Animals, particularly cats, can exist with chronic, severe anaemia for weeks to months without many clinical signs. So an animal that presents with severe clinical signs with only a moderate anaemia likely has an acute onset of anaemia, usually due to haemorrhage or haemolysis.

There are three basic mechanisms of anaemia:

1) decreased RBC production by the bone marrow
2) RBC loss from the body i.e. external haemorrhage
3) RBC destruction i.e. haemolysis

However this is complicated by the fact that the spleen contains 20-30% of the erythroid mass, with splenic contraction able to release significant RBC numbers into the circulation.

An animal's hydration status needs to be assessed before interpreting the PCV. This can be done crudely by determining the total plasma protein (TPP). Other factors can however affect the TPP, such as protein loss via haemorrhage, intestinal or glomerular disease. Albumin and globulin fractions are also helpful in determining the presence of other disease with glomerular disease likely to cause albumin loss only.

Determining Erythroid Regeneration

Quantifying reticulocytes in the peripheral blood is the most consistent way to evaluate regeneration. Determining whether the anaemia is regenerative can be helpful in determining the cause, with regeneration indicating bone marrow response, usually due to haemorrhage or haemolysis.
There may be non-regenerative anaemia for the first 3 to 5 days after haemorrhage or haemolysis, as this is the time it takes for aggregate reticulocytes to be released into the circulation once erythropoietin has stimulated the bone marrow. Bone marrow reticulocyte production can be stimulated up to 6-8 x normal in dogs and 3-5 x normal in cats. The reticulocyte response is generally proportional to the degree of the anaemia. A mild anaemia may not stimulate reticulocytosis, with the bone marrow response likely to be with mature RBCs.

Reticulocytes have ribosomes (ribonucleic acid, RNA) for continued haemoglobin synthesis and these appear as blue granules when stained with new methylene blue.
Absolute reticulocyte count is a more consistent indicator of bone marrow production, with some automated haematology machines such as the the LaserCyte™, generally giving reliable results in the author's opinion.

Absolute reticulocyte counts of > 80 000 /uL for dogs and > 60 000 /uL for cats generally indicating adequate regeneration.
If reticulocyte percentages are given, these need to be corrected for the level of anaemia, with > 1.5% indicating regeneration.
In cats, reticulocyte counts need to be based on just the aggregate reticulocytes and not the punctuate reticulocytes, which can persist in the circulation for up to 12 hours and don't accurately reflect the regenerative response.

Blood smear examination
This should be standard part of investigation into anaemia. These can be sent to the reference laboratory; however the practitioner can get a good feel for regenerative response with repeated blood smear examinations. Unexpected results on haematology, particularly in-house haematology, can be checked on the blood smear, with the advantage of being able to assess red and white cell morphology as well as platelet numbers and/or clumping.
A hospitalised IMHA patient can be monitored for regeneration with a daily PCV/TPP and in house blood smear examination.

Red Blood Cell Indices/Smear Morphology
RBC morphology should be evaluated on the thick edge of the monolayer area. Canine RBCs should display normal central pallor in the proper areas of the smear.

Polychromasia
Denotes an increase in polychromatophils (these are reticulocytes), with slightly bluer cytoplasm and larger than mature RBC

Macrocytosis
Increased mean corpuscular volume (MCV). Reticulocytosis is the primary cause of this, with regenerative anaemias typically macrocytic and hypochromic.
Other causes of macrocytosis:

1) artifactual swelling of rbcs in EDTA during prolonged storage
2) breed e.g. poodles
3) FeLV in cats

Anisocytosis
Variation in RBC size – commonly caused by regenerative anaemia and release of immature macrocytes.
Red blood cell distribution width (RDW) is a number derived from automated haematology counters to describe the amount of anisocytosis.

Other haematologic findings that may be found in regenerative anaemia include **Howell-Jolly bodies**, **nucleated red blood cells (NRBCs)** and **basophilic stippling**.
NRBCs may be released in regenerative anaemia or may be independent of erythropoeisis, for example in splenic disease, extramedullary haematopoiesis (EMH), lead poisoning, hyperadrenocorticism, leukaemia and other bone marrow diseases.
Basophilic stippling may be seen in regenerative anaemia or lead poisoning.

Spherocytes & Autoagglutination
Moderate-to-abundant spherocytes or autoagglutination, or both indicate IMHA.

To identify spherocytes, examine the monolayer area where any normal RBCs are lying flat and have central pallor. **Spherocytes** appear smaller in diameter and darker orange, and they lack central pallor. If Erythrocytes aren't completely phagocytosed, their membrane may be partially internalised, followed by resealing of the membrane and reentry of the remaining red cell into circulation as a spherocyte.

Autoagglutination is immune-mediated aggregation of RBCs into grape-like clusters.
Autoagglutination may be considered a biological Coomb's test. RBCs coated with high titre of antibody and complement can spontaneously agglutinate. Both autoagglutination and strong **rouleaux** may be visible grossly in the blood tube. Rouleaux formation is like stacking of coins, with increased rouleaux usually indicating inflammatory disease, due to increased fibrinogen and gamma globulins. Autoagglutination and rouleaux can be differentiated by the addition of saline, with saline causing dispersion of rouleaux.

Red Blood Cell Fragmentation
RBC fragmentation may be the result of metabolic disorders, intravascular trauma or Fe deficiency anaemia. D.I.C. causes fibrin strands in the blood flow that can split RBCs. Small, irregular RBC

fragments are called **shistocytes, keratocytes, helmet cells or RBC fragments. Acanthocytes,** which have a few irregular projections are also associated with RBC fragmentation or hepatic disease.

REGENERATIVE ANAEMIAS

Blood Loss Anaemia

i. External blood loss
ii. Internal blood loss
iii. Iron deficiency anaemia

Haemolytic Anaemia

i. Immune-mediated haemolytic anaemia (IMHA)
ii. Cold haemagglutin disease
iii. Blood parasites – Mycoplasma haemofelis, Mycoplasma haemocanis, Babesia canis, (Cytauxzoon felis)
iv. Heinz body anaemia and methaemoglobinaemia
v. Zinc or copper toxicity
vi. Hypophosphataemia
vii. Hereditary haemolytic anaemia – Pyruvate Kinase (PK) deficiency, Phosphofructokinase (PFK) deficiency

NONREGENERATIVE ANAEMIAS

Secondary Anaemia

i. Anaemia of inflammatory disease
ii. Anaemia of chronic renal disease
iii. Anaemia of chronic hepatic disease
iv. Hypothyroidism
v. Hypoadrenocorticism

Iron Deficiency Anaemia

Bone Marrow Disorders

Aplastic pancytopaenia
Myelofibrosis
Pure red blood cell aplasia (PRCA)
Myelodysplastic syndromes
Leukaemia
Haemophagocytic syndromes

Drug-Induced Haematologic Dyscrasia

Oestrogen toxicity
Sulfadiazine toxicity
Phenylbutazone toxicity

Infections

FeLV, FIV, Feline panleukopaenia virus
(Ehrlichia)

REGENERATIVE ANAEMIAS

BLOOD LOSS ANAEMIA

External blood loss is often obvious from the history or physical examination, however blood loss into the GIT or internal blood loss into the thorax or abdomen may be occult.
GIT bleeding may present as ***haematochezia*** (fresh blood in the faeces) or ***melaena*** (black, tarry stools). Tests for occult blood are notoriously unreliable, mostly because of false positives. Myoglobin

in meat diets will frequently cross react with a faecal occult blood test so a patient needs to be put on a fish or white meat based diet for a few days before performing the faecal occult blood test.
In practice, if blood loss is still suspected it can generally be narrowed down to the GIT by exclusion, using a combination of abdominal or thoracic ultrasound +/- fluid centesis, thoracic radiography or even a blind diagnostic peritoneal lavage. Always assess the PCV/TPP of sanguinous effusions. Haematology can be quite variable after blood loss depending on duration, severity and frequency of haemorrhage.
In haemolytic anaemia and ***internal blood loss***, PP concentration tends to be normal or may even be slightly increased as no protein is lost from the body. Trends in PP need to be followed and this and PCV can be substantially affected by hydration status.

External Blood Loss
PCV in adult dog will take up to 3 days to fully reflect the severity of the blood loss until fluid volume expansion occurs. Splenic contraction occurs in the first few hours after haemorrhage, increasing the PCV.
Reticulocytes should be present 3 days after haemorrhage with peak reticulocyte release 4 - 5 days post-haemorrhage. PCV initially increases rapidly over the first two weeks but may only slowly increase when there is little remaining hypoxia to stimulate erythropoietin.
Chronic haemorrhage over several weeks causes ***iron deficiency*** and a negative protein balance, impairing erythropoiesis and causing increasingly weaker regenerative responses. A blood loss anaemia may be initially pre-regenerative, then regenerative, progressing to poorly regenerative or nonregenerative due to Fe deficiency.
Puppies or kittens are usually undergoing maximum erythropoiesis and can rapidly become iron depleted due to blood loss e.g hookworms, coccidia.

External blood loss will generally cause a low to low-normal TPP, however in internal blood loss or haemolysis the TPP may be normal or even increased. PP is replaced by the liver and lymphoid tissues more quickly than the bone marrow can replace RBCs.
If TPP is low due to haemorrhage, albumin and globulin fractions should be equally reduced, however protein losing enteropathies will also cause this pattern. Haemolysis, reducing PCV, and a PLE may also in theory replicate the haematology pattern seen with haemorrhage. Remember, a protein losing nephropathy will only cause a reduction albumin and can be assessed by urine protein measurement.

In ***feline*** cases of anaemia, moderately to markedly increased ***aggregate*** reticulocytes with few punctate indicate a recent anaemia (e.g. 2 to 4 days). Increased ***punctate*** reticulocytes without increased aggregate indicate only mild anaemia or anaemia of 1 to 3 weeks duration.

HAEMOLYTIC ANAEMIA

This is usually identified by a markedly regenerative anaemia without hypoproteinaemia or other evidence of blood loss.

Extravascular haemolysis results from phagocytosis of RBCs by macrophages in the spleen, liver, and bone marrow. This occurs much more frequently than intravascular and is often associated with splenomegaly and hepatomegaly.

Intravascular haemolysis indicates an acute, severe anaemia and may be accompanied by haemoglobinuria, haemoglobinaemia and possibly lysed RBCs (ghost cells) on the blood smear. Extravascular haemolysis will also be occurring concurrently. Heinz body anaemias and complement fixing immune mediated anaemias are the most common causes of intravascular haemolysis.

Icterus occurs with intravascular and extravascular haemolysis. Increased unconjugated bilirubin from RBC lysis exceeds the liver's excretion capacity, however liver hypoxia or toxic damage may reduce bilirubin metabolism and cause cholestasis, increasing the amount of conjugated bilirubin in the circulation.

Immune-Mediated Haemolytic Anaemia

IMHA is a type II hypersensitivity reaction. Extravascular haemolysis occurs when immunoglobulin (Ig) or complement coated RBCs are removed by the mononuclear phagocytic system (MPS). Phagocytosis of opsonized RBC occurs following binding of the fragment crystallizable or constant fragment (Fc) of the immunoglobulin molecule to Fc receptors on cells of the mononuclear-phagocytic system.

If enough IgG or IgM molecules coat the RBCs to enable complement fixation, intravascular haemolysis may result (10-20% of canine cases). Antibody binding can initiate complement fixation and formation of a transmembrane pore (membrane attack complex) with resultant intravascular osmotic hemolysis.

Antibodies of the IgG subclass, alone or in combination with IgM, are the most commonly identified erythrocyte-bound immunoprotein in canine IMHA. Newer flow cytometric techniques have enabled more accurate identification of the Ig subclass involved in IMHA.

IgG antibodies are relatively poor activators of the classical complement pathway and IgG-sensitized RBCs are generally eliminated through extravascular hemolysis. IgM-sensitized RBCs are generally eliminated through a combination of intravascular hemolysis (due to IgM-mediated activation of the classical complement pathway) and extravascular phagocytosis.

Primary IMHA is a true autoimmune reaction against RBCs, with 60-75% of cases thought have this form, with no underlying causes identified. Erythrocyte glycophorins, RBC anion channel band 3, and the cytoskeletal protein spectrin have been implicated as autoantigens in canine IMHA
Secondary IMHA is more common in cats and occurs when RBCs are destroyed as "innocent bystanders" in an immune reaction against a foreign protein which may have coated the RBCs. The triggering protein may be secondary to viral or bacterial infection, drugs or neoplasia.

Clinical Presentation
Signalment
IMHA usually occurs in middle aged dogs, with a median age of 6 – 7 years. Any breed may be affected, however Cocker Spaniels, Springer Spaniels, Collies, Poodles, Old English Sheepdogs and Irish Setters have been overrepresented in studies. Most studies have shown a female predominance. One study of IMHA in cats reported a median age of 2.

Clinical Signs
Clinical signs of IMHA reflect the inflammatory reaction and anaemia. The most common clinical signs are anorexia, lethargy, pallor, weakness, icterus, tachycardia and hepatosplenomegaly.
Less commonly reported signs are vomiting and diarrhoea, or sudden collapse and syncope. Owners may report discoloured urine, which is usually bilirubinuria, or less commonly haemoglobinuria in cases with intravascular haemolysis.
A haemic murmur may be identified in dogs with severe anaemia. Petechiae may be present in cases with concurrent thrombocytopaenia or vasculitis. Fever and lymphadenopathy are sometimes present.

Laboratory Results
PCV at presentation can be as low as 6 and up to half of all IMHA dogs have poorly regenerative or non-regenerative anaemia and 11 of 19 cats were non-regenerative.
Spherocytes are common, and in those that are regenerating, polychromasia, anisocytosis, macrocytosis and nucleated RBCs are commonly present. Microscopic autoagglutination may be noted in the blood collection tube or on blood smears.
Leukopaenia is uncommon and may result from immune-mediated neutropaenia, sepsis or primary bone marrow disease.
Moderate to marked leukocytosis is much more commonly noted, with inflammatory cytokines acting as colony-stimulating factors and bone marrow activation in response to anaemia. Centrilobular hepatic necrosis secondary to hypoxaemia has been reported to contribute to the leukocytosis.
Thrombocytopaenia is a concurrent finding in approximately 70% of dogs and 42% of cats with IMHA. This may be due to immune mediated destruction (Evan's syndrome), or consumption in the case of sepsis or DIC.

Biochemical changes may reflect haemolysis, dehydration and hypoxic organ damage.
There are no consistent biochemical abnormalities in cases of IMHA, although two thirds or more may have hyperbilirubinaemia with bilirubinuria.
Prerenal azotaemia may be seen in severely affected cases. As previously mentioned, hepatic hypoxaemia may be seen, causing mild to moderate elevations in hepatic transaminases.

Direct Coomb's Test
The direct Coomb's test, usually a polyvalent direct antiglobulin test, detects antibodies or complement on the RBC surface. 35-60% of IMHA cases have a positive Coomb's test result.
False positives may be due to concurrent disease states such as neoplasia, particularly lymphoproliferative disorders, bacterial infections, infection with erythrocyte parasites, drugs, acute or delayed transfusion reactions or neonatal isoerythrolysis, vaccines, bee stings or non-specific adsorption of serum proteins to the erythrocyte surface.
False negatives may be due include low level of membrane-bound immunoglobulin or complement, low binding affinity or high dissociation constant of membrane-bound antibody, previous corticosteroid therapy, and technical error. The length of time a sample can be stored in EDTA and still give an accurate Coomb's result is also unknown.

Ancillary diagnostics
Arterial blood gas is indicated in the dyspneoic dog. Pulmonary thromboembolism may cause profound hypoxaemia (markedly increased alveolar-arterial (A-a) oxygen gradient) with normocapnia.

Coagulation parameters in IMHA patients have been intensely studied in recent years. Prolonged APTT and PT have been identified in a large proportion of IMHA patients, with many fulfilling the diagnostic criteria for DIC, including increased fibrinogen, fibrinogen degradation products (FDPs) and D-dimer concentration.
A hypercoagulable state has long been suspected in IMHA patients which has been supported by studies, including recent flow cytometric work.

Bone marrow cytology or histopathology may be indicated, particularly in those with a poor regenerative response. Most cases will show erythroid hyperplasia, however erythrophagocytosis or erythroid hypoplasia may indicate immune mediated destruction of RBC precursors.

Thoracic radiographs are often utilised to rule out underlying neoplasia or infection that may be causing secondary IMHA. Pulmonary thromboembolism can sometimes be detected as a pronounced interstitial pattern, patchy alveolar pattern or even pleural effusion.

Abdominal ultrasonography (or radiographs) will often show hepatosplenomegaly but may identify abdominal neoplasia or even gastrointestinal foreign bodies.

In the author's practice, a standard workup of a stable IMHA patient will involve:

1) Haematology and blood smear analysis (often submitted to an external reference laboratory)
2) Slide autoagglutination test +/- Coomb's test
3) Full biochemistry
4) Abdominal ultrasound
5) Thoracic radiographs (three views)

An unstable IMHA patient may have in addition (usually with oxygen supplementation and/or blood transfusion as indicated):

6) Blood pressure(s)
7) Blood gases
8) Urinalysis
9) Coagulation tests – PT, APTT

Bone marrow aspirates/biopsies are not usually performed unless there is a lack of regenerative response after 4 to 5 days or if there is an older patient and bone marrow neoplasia is strongly suspected.
Treatment is usually initiated, as discussed below, and daily PCV/TPP and blood smear analysis performed, with a complete blood count often every 48 hours.

Therapy for IMHA

Supportive Care
Initial supportive care involves the maintenance of hydration, acid-base balance and organ perfusion. Diuresis may be indicated in dogs with intravascular haemolysis to prevent haemoglobin nephrotoxicosis. Dogs may present severely hypotensive and/or in acute renal failure, requiring aggressive fluid therapy, unless cardiovascular compromise is apparent. Intravenous catheters have been shown to be a risk factor for PTE, and unnecessary catheterisation is to be avoided. Strict aseptic technique needs to be observed particularly seeing these patients are about to be aggressively immunosuppressed.
H_2 receptor antagonists such as ranitidine or proton pump inhibitors such as omeprazole are often initiated early to attempt to avoid gastrointestinal ulceration.

Blood transfusion
The indications and techniques of blood transfusions will be covered in a later lecture, however it is important to treat the patient, not the PCV and carefully follow trends in PCV. Clinical parameters such as attitude, exercise tolerance, heart rate and respiratory rate need to be monitored closely. Autoantibodies in IMHA patients may shorten transfused RBC survival and may suppress the patient's erythropoietic response. Transfusions may also increase the risk of PTE.

Immunosuppressive Therapy

Glucocorticoids

The mechanisms by which glucocorticoids are effective in treating IMHA have not been proven but are suspected to be:

1) rapid impairment of macrophage-mediated erythrophagocytosis

2) delayed (within 14 days) decrease in immunoglobulin concentration

3) altered T lymphocyte generation and function secondary to decreased interleukin (IL)-6 production

Initial treatment is usually with **prednisolone** (2 – 4 mg/kg/day), or occasionally **dexamethasone** (0.25 to 0.5 mg/kg/day). This dose needs to be maintained for 5 to 7 days. At this point the dosage can be reduced by 25 to 50% every 3 to 4 weeks if appropriate. If at any point there is a reduction in PCV or any autoagglutination, the dose reduction needs to be reversed, or possibly full immunosuppressive doses reinstituted. When the dose of prednisolone is reduced to 0.5mg/kg/day, alternate day therapy can be instituted.

Adverse side effects:

1) polyuria, polydipsia

2) polyphagia

3) excessive panting

4) steroid myopathy

5) gastric ulceration

6) vacuolar hepatopathy

7) iatrogenic hyperadrenocorticism

8) cutaneous atrophy

9) alopecia

10) suspected clinical risk factor for thromboembolic disease

Azathioprine

In recent years there has been a trend toward combination immunosuppression in canine IMHA. Due to its association with increased survival time, azathioprine has become the primary adjunctive immunosuppressive agent for canine patients with IMHA.

Azathioprine is a purine analogue antimetabolite, inducing immunosuppression by impairing RNA, DNA and protein synthesis, thereby inhibiting lymphocyte proliferation. Azathioprine therapy may take up to a week or more to significantly inhibit lymphocyte proliferation. Azathioprine does not affect peripheral lymphocyte count or serum immunoglobulin concentrations.

The recommended initial dose of azathioprine in canine IMHA is 2 mg/kg q24h. Some recommend alternate day dosing once prednisolone therapy has been tapered to every other day while others recommend a transition to alternate day therapy within 4 to 7 days.

Side effects (these are uncommon):

1) acute pancreatitis

2) bone marrow toxicosis

3) hepatotoxicosis

4) gastrointestinal illness

Ciclosporin (cyclosporine)

Ciclosporin binding of the intracellular protein cyclophilin and the resulting inhibition of calcineurin, prevent nuclear translocation of a family of proteins (nuclear factor of activated T cells) essential for transcription of the IL-2 gene. The absence of IL-2 prevents further activation and proliferation of T lymphocytes and secondarily results in IL-4, interferon-γ, and granulocyte colony-stimulating factor suppression, but does not affect peripheral lymphocyte count or serum immunoglobulin concentrations.

No prospective trials have reported on the efficacy of ciclosporin in dogs with IMHA. Its current use appears to be in patients with severe clinical presentations or dogs that fail to respond to standard therapy. Some clinicians have used ciclosporin as initial therapy, before the cheaper azathioprine becomes effective.

There appears to be wild variation in the gastrointestinal absorption and pharmacokinetics of cyclosporine, with unpredictable responses between dogs and even in an individual dog. The initial recommended dose of ciclosporin for management of canine IMHA is 5 to 10 mg/kg administered orally once or twice daily. The author's clinic usually starts on 2.5 mg/kg twice a day. It is important that the microemulsion is used, either the human preparation Neoral™ or the veterinary Atopica™. Unfortunately these products are still very expensive and remain all but cost prohibitive for large dogs.

Monitoring of trough plasma concentrations every 2 to 4 weeks to maintain concentrations between 100 to 300 ng/mL has been suggested, although there is no general consensus on this. Strategies to increase absorption and reduce costs include dosing with grapefruit juice or with ketoconazole.

Side effects;

1) gastrointestinal illness (specifically vomiting), dosing with food may reduce this

2) gingival hypertrophy

3) papilloma-like skin lesions

4) hair loss

5) increased susceptibility to opportunistic infections and cancer are accepted complications in humans

Leflunomide, mycophenolate mofetil, and liposomal-encapsulated clodronate are among the novel immunosuppressive and immunomodulating agents to be investigated in canine IMHA.

Leflunomide and mycophenolate mofetil inhibit *de novo* pyrimidine and purine biosynthesis respectively, thereby inhibiting DNA synthesis, reducing lymphocyte and antibody production. Liposomal-encapsulated clodronate clodronate leads to clodronate being preferentially phagocytosed by splenic macrophages leading to macrophage depletion.

Leflunomide has not been critically evaluated, however has been reported effective in cases series of immune mediated diseases such as IMHA and immune mediated polyarthritis, with minimal side effects. A recent case report of a diabetic dog successfully treated with human intravenous immunoglobulin and leflunomide without any other immunosuppressive drugs including glucocorticoids. Leflunomide, however, remains cost-prohibitive at this stage.

Human Intravenous γ Globulin

Human IVIG is thought to block Fc receptors on macrophages and possibly downregulating of autoantibody production leading to reduced RBC destruction. Case series have reported good results, often with refractory cases of IMHA or immune mediated thrombocytopaenia.

When given at a dose of 1 g/kg intravenously over 6 to 12 hours, a profound reticulocytosis occurs followed more slowly by a rise in PCV. This product can be difficult to obtain, is very expensive, and being a human blood product, introduces some occupational health and safety risks.

Thromboprophylaxis

Thromboembolism is an important complication and the main cause of death from canine IMHA; however, no prophylactic anticoagulant has been shown to be effective.

Treatment with *unfractionated heparin* has not associated with prolonged survival. Fractionated, or *low molecular weight heparin*, inhibiting factor Xa may be effective but there is such variable pharmacokinetics in animals. Doses up to 300IU/kg q6h did not reach target anti-Xa activity in one study, well over the 150IU/hg q12h dosing commonly used. The substantial costs involved limit their use.

Activated platelets have been identified in IMHA platelets. Studies have shown improved short- and long-term survival in dogs with IMHA treated with *aspirin* (0.5 mg/kg/day). Aspirin inhibits cyclooxygenase-mediated synthesis of thromboxane A_2, a powerful stimulant of platelet aggregation. Aspirin can be used in doses up to 1-2mg/kg/day.

Heinz Body Anaemia

Many substances oxidize haemoglobin, causing it to precipitate and form Heinz bodies, methaemoglobin, or both. They may appear as lighter coloured, single round bodies within RBCs or

may bulge from the cell surface. Canine Heinz bodies are not normally present and may be small, irregular and multiple. Eccentrocytes result from oxidative damage to RBCs, with onion toxicity being the most common cause in dogs.

Canine Heinz Body Anaemia, causes:

1) Onions
2) Naphthalene
3) Propylene glycol
4) Copper, zinc
5) Paracetomol

Feline Heinz Body Anaemia

Both healthy and ill cats frequently have Heinz bodies, with feline haemoglobin m ore susceptible to oxidative damage and the feline spleen being less efficient in removing damaged RBCs from the circulation. Causes in cats are:

1) Paracetamol
2) Propofol
3) Propylene glycol
4) Salmon based diets
5) Diabetes mellitus
6) Hyperthyroidism
7) Renal failure
8) Lymphosarcoma

NON-REGENERATIVE ANAEMIAS

Non-regenerative anaemias are usually mild, normocytic, normochromic, secondary to systemic diseases and usually lack diagnostic changes in RBC morphology.

Diagnostic Approach

If leukopaenia or thrombocytopaenia i.e. pancytopaenia or bicytopaenia are present in addition to non-regenerative anaemia. This usually indicates primary bone marrow disease, and a bone marrow aspirate or biopsy is usually indicated.

Severe nonregenerative anaemias are often associated with non-regenerative IMHA or pure RBC aplasia.

Microcytic, hypochromic anaemia usually indicates iron deficiency.

Macrocytic normochromic anaemia without reticulocytosis in cats usually indicates FeLV-induced myeloproliferative disorders.

Secondary Anaemias

Anaemia of Inflammatory Disease (AID)

This is usually mild to moderate normocytic, normochromic, with low serum iron and increased bone marrow and tissue iron. During inflammation, macrophages release inflammatory cytokines, including IL-1, IL-6 and TNF. These inflammatory cytokines cause iron to be sequestered into macrophages, reducing serum iron.

The anaemia is initially normocytic and normochromic, but may progress to microcytic and hypochromic in dogs. Serum iron should be low-normal to low, but in contrast to iron deficiency, AID is supported by documenting low-normal to decreased transferrin, and high-normal to elevated ferritin. Bone marrow biopsy should reveal normal to mildly depressed erythropoiesis with normal to increased iron in dogs.

Anaemia of Chronic Renal Disease

Erythropoietin (EPO) is produced mainly by peritubular fibroblasts of the **renal cortex.** Regulation is believed to rely on a feed-back mechanism measuring blood oxygenation. Erythropoietin has its primary effect on red blood cells by promoting red blood cell survival through protecting these cells from **apoptosis**, with the colony forming unit-erythroid **(CFU-E)** completely dependent on erythropoietin. Under hypoxic conditions, the normal kidney will produce and secrete erythropoietin to increase the production of red blood cells by targeting CFU-E.

The mechanism of anaemia in chronic renal disease is multifactorial, involving more than just a relative deficiency of EPO. Other factors believed to be involved include ineffective erythropoiesis, a shortened RBC lifespan and gastrointestinal blood loss.

Anaemia of Chronic Hepatic Disease

This may have multiple causes, including anaemia of inflammatory disease (AID). Abnormal lipid metabolism may cause altered RBC shapes (*acanthocytes*) and a shortened RBC life span.
Cats with hepatic lipidosis or inflammatory liver disease frequently have marked *poikilocytosis.*
Reduced hepatic synthesis of coagulation proteins may cause haemorrhage.
Decreased hepatic function may lead to deficiency in nutrients for erythropoiesis. Microcytosis is common in dogs with portosystemic shunts and can lead to a 'functional' iron deficiency.

Hypothyroidism and Hypoadrenocorticism
The anaemia secondary to endocrine disease is usually mild and clinically insignificant.
Anaemia in *hypothyroidism* is considered an adaptation to decreased oxygen demands due to decreased basal metabolic rate, and thus is a consequence, rather than cause, of lethargy. Thyroid hormone normally stimulates burst-forming units–erythroid and colony-forming units–erythroid directly and indirectly via EPO.
Glucocorticoids enhance erythropoiesis and this is part of a normal stress response. It is thought they stimulate glucocorticoid receptors on erythroid progenitor cells. Gastrointestinal ulceration in hypoadrenocorticism may cause acute haemorrhage.

Iron Deficiency Anaemia
Iron deficiency in a mature dog or cat is due to chronic blood loss. Causes include:

i) severe flea infestation
ii) chronic haematuria
iii) chronic gastrointestinal bleeding, mostly neoplasia

The anaemia is usually non-regenerative or poorly regenerative, microcytic and hypochromic. Microcytosis develops because metarubricytes stay in the bone marrow longer, undergoing an additional cell division, while awaiting completion of Hb synthesis.
Iron deficiency is further supported by documenting low to low-normal total serum iron, and high-normal to elevated transferrin (total iron-binding capacity) with low saturation. Serum iron and transferrin are measurable in most reference laboratories. Serum ferritin is more reliable, and should be low-normal to low, but must be measured by a validated species-specific assay and is less available. Remember AID is supported by documenting low-normal to decreased transferrin, and high-normal to elevated ferritin.

Drug-Induced Haematologic Dyscrasias
Drugs reported to cause bone marrow dyscrasias in dogs include oestrogens, phenylbutazone, sulfonamides, cephalosposrins, chemotherapeutic agents, thiacetarsamide and phenobarbitone.
In cats they include chemotherapeutic agents, chloramphenicol, griseofulvin and methimazole.

Infections
FeLV, FIV, parvovirus, (ehrlichiosis)

SEVERE Non-Regenerative Anaemia

Nonregenerative IMHA
IMHA is classically characterized by peripheral destruction of red cells and a strong regenerative response. Less commonly, immune-mediated destruction of erythroid precursors in the bone marrow can occur instead of, or in addition to, the peripheral RBCs.
Depending on the level and extent of erythroid precursor destruction, bone marrow may have erythroid hyperplasia or erythroid hypoplasia, and in some cases 'pure red cell aplasia' (PRCA). In some dogs a 'maturation arrest' is present, with more mature cells e.g. metarubricytes, late stage rubricytes.
Immune-mediated erythroid hypoplasia and related disorders are presumptively diagnosed on the basis of ruling-out other causes of erythroid hypoplasia, supportive bone marrow biopsy findings, and on response to immunosuppression (which may or may not be successful). The diagnosis is also supported by other concurrent immune-mediated disorders (e.g., polyarthritis), positive antinuclear antibody titer (ANA), positive Coombs' test, and spherocytes or presumed immune mediated thrombocytopaenia.

Myelodysplastic Syndrome (MDS)
MDS is characterized by peripheral blood cytopaenias, but normal to increased marrow cellularity with dysplastic changes, and a risk of progression to acute myeloid leukaemia (AML). Myeloid leukemia refers to neoplasia arising from haematopoietic cells in the bone marrow, be they of erythropoietic, thrombopoietic, granulopoietic, monocytopoietic, mixed-lineage, or pluripotent stem cell origin, with or without overt peripheral blood involvement.
The classification and nomenclature of MDS and AML are evolving. What these syndromes have in common an abnormal clone of hematopoietic progenitor cells that may replace normal hematopoietic tissue. The hematologic and clinical progression is highly variable.

Myelophthisis
Erythroid cells may be 'crowded out' by metastatic cancer cells, nonmyeloid hematopoietic neoplasia (acute lymphoid leukemia, multiple myeloma, mast cell neoplasia), granulomatous inflammation (e.g., histoplasmosis), or myelofibrosis.
In addition to physical displacement, neoplastic cells may depress erythropoiesis by molecular mechanisms, such as secreting 'anaemia inducing substance', AIS.
Myelofibrosis is a nonspecific finding, causes include:

i) idiopathic
ii) FeLV infection in cats
iii) chronic bone marrow inflammation e.g. IMHA
iv) leukemia

Anaemia and Cancer

Anaemia is one of the most common paraneoplastic syndromes in dogs and cats, occurring in a wide range of neoplasms. In a 2009 paper, 32% of dogs with lymphoma were found to be anaemic, and this was found to be predictive of poor outcome.

Cancer can cause anaemia by many different mechanisms including blood loss, bone marrow infiltration, AID, IMHA, hyperoestrogenism, and microangiopathic haemolytic anemia.

Lymphoma, leukemia, malignant histiocytosis, and multiple myeloma are examples of neoplastic diseases that can lead to anaemia via marrow infiltration.

There may also be blood loss secondary to erosion of underlying tissue and vessels e.g. nasal carcinoma and hemangiosarcoma, and erythrophagocytosis by tumour cells, as with malignant histiocytosis.

Haemophagocytic syndromes can be idiopathic or occur secondary to IMHA, IMT, MDS, histiocytic disorders or neoplasia.

Dr Karina Graham
Internal Medicine

Blood transfusion medicine

Dr Karina Graham

Blood transfusion therapy is a vital component of veterinary medicine. The aim of this presentation is to provide general practitioners with the essential knowledge and techniques to allow confident administration blood products safely and to the right patient. There are many products available throughout the world, however fortunately or otherwise, Australia (and probably New Zealand) is lagging behind. Australian's have whole blood, packed red blood cells and plasma available making the selection relatively easy.

Melbourne canine blood back can courier packed red cells ($300) or plasma ($185) to practitioners in most major cities in Australia usually within 24hrs. The limitations include availability of products and practice location. All practitioners should have access to donor animals by recruiting staff or client-owned pets.

There are several indications for blood component transfusions and it basically comes down to what's in the product that your patient needs. Availability and cost are often contributing factors.

Whole blood contains blood and the anticoagulant. One unit is equal to 450ml +/- 45ml blood in 63ml of anticoagulant. Once refrigerated, the white blood cells and platelets become non-functional. Dosage is approximately 10-22ml/kg. It can be stored in the fridge for approximately 20 days. Major indications include blood loss, severe life threatening thrombocytopenia, and coagulopathies.

Packed red blood cells contain the cells and a small amount of plasma and anticoagulant. They don't contain platelets or clotting factors. If 450ml of whole blood is collected, the packed red blood cells volume is about 200mls. Dosage is 6-10ml/kg. They can be stored in the fridge for 5 weeks. Indications include clinically significant haemolytic anaemias or haemorrhage.

Fresh frozen plasma is prepared by centrifuging whole blood and anticoagulant and freezing with 8hrs. The anticoagulant remains with the plasma. It contains all clotting factors and remains viable for 6 months in a -20^{o}C standard freezer. Indications include rodenticide toxicity, other coagulopathies and pancreatitis. It should not be used as a source of albumin, volume expansion or nutritional support. Dosage 6-10ml/kg.

Canine blood groups

DEA 1.1* DEA 1.2* DEA 1.3 DEA 1
DEA 3
DEA 4 – most common
DEA 5
DEA 6
DEA 7*
DEA 8
Dal

*most significant in producing clinical transfusion reactions

Dog Erythrocyte Antigen (DEA) is the name given to a molecule that sits on the erythrocyte membrane. It can be one of approximately 10 different types. In the dog, naturally occurring alloantibodies to various DEAs are rare. This means that you can transfuse a dog without knowing the blood type of the donor or recipient reasonably safely – HOWEVER, it is the subsequent transfusions that are potentially dangerous. For example, a recipient that is negative for DEA 1.1 that has previously received DEA 1.1 positive blood will likely have antibodies against DEA 1.1. That means a repeat transfusion of DEA 1.1 positive blood will likely result in a haemolytic transfusion reaction. This has also been described for DEA 4, DEA 7 and proven in a laboratory setting for DEA 1.2. It has therefore

been recommended to select donors that are negative for DEA 1.1, DEA 1.2 and DEA 7. A "universal donor" is one that is DEA 1.1 negative, 1.2 negative, 3 negative, 4 positive, 5 negative and 7 negative. DEA 4 positive it thought to be safe because of the high prevalence of DEA 4 positive dogs (98%) and its inability to produce dramatic transfusion reactions. Realistically in Australia, a DEA 1.1 negative dog is considered universal. You will be surprised to know that the Melbourne canine blood bank does not perform blood typing on any of their donor dogs...

Feline blood groups

There are 3 blood types in cats: A, B and AB. Cats do have naturally occurring alloantibodies to type A and B, therefore they are at high risk for a transfusion reaction with every transfusion. It is mandatory to perform blood typing on donor and recipient cats. Type B cats have strong haemagglutinating IgM antibodies to type A blood which means you are risking an acute haemolytic crisis and death in type B cats given type A blood. The reverse is also true, however the antibodies are somewhat "weaker". Historically, it was thought that 99% of cats are type A and so the risk is relatively low because all the donors are likely to be type A. This is data from the USA and DOES NOT hold true for Australia. Up to 73% of Australian domestic short hair cats are type B. Furthermore, there are known breed associations. The Bristish Shorthair and Devon Rex are reported to have the highest incidence of type B individuals (~50%) and ALL Siamese, Oriental shorthair, Burmese, Tonkinese, American shorthair and Norwegian forest cats are reported to be type A.

Anticoagulants

Blood must be collected into an anticoagulant. An anticoagulant like heparin or 3.8% sodium citrate does not contain nutrients to preserve red cell function and so must be used for immediate transfusion use. Anticoagulant preservative like citrate phosphate dextrose adenine (CPDA-1) contains nutrients and allows storage, at 0-6C, for up to 20 days. CPDA-1 is found in most commercially prepared multiple-bag systems. In our clinic, they come in packs of 10 collection bags. One bag is sacrificed and the contents drawn up into a 60ml syringe for feline blood collection.

Anticoagulant	**Ratio with blood**	**Storage time**
Heparin	625 U / 50ml blood	For immediate use
3.8% sodium citrate	1ml / 9ml blood	For immediate use
CPDA-1	1ml / 7ml blood	20 days

Pretransfusion compatibility testing

Dogs: blood typing and cross-matching are not routinely performed before the first transfusion of DEA 1.1 negative donor blood. Blood typing or cross-matching is not required before transfusion of canine plasma. All subsequent blood transfusions should be cross-matched at a minimum.

Cats: All cats must be blood typed. Cross-matching can be used if blood typing is not available. The simplified typing cards (Rapid Vet-H) for cats are easy and relatively inexpensive.

Cross matching

Equipment:
1ml EDTA blood from recipient
1ml EDTA blood from potential donor
Tabletop centrifuge
3ml test-tubes (sterility not required)
0.9% saline
Disposable pipettes
Test tube rack

Procedure:

1. Obtain EDTA blood from the recipient and the potential donor or the tube segments of blood from the units being considered for transfusion
2. Centrifuge both donor and recipient blood for 5 minutes at 1000g
3. Using pipettes, remove the plasma and save in separate labelled tubes
4. Wash the red blood cells by adding saline to the red cells to fill the tube. Resuspend the red cells in the saline by tapping the bottom of the tube with a finger
5. Centrifuge the red cells and saline for 5 minutes at 1000g. Pipette off saline and discard
6. Repeat steps 4 and 5 twice
7. After third washing of the red cells in saline, resuspend the red cells to a 3% to 5% solution. It will appear bright cherry red.
8. For each potential donor, mix two drops of recipient plasma and one drop of donor red cell suspension for the major crossmatch. Mix gently
9. For each potential donor, mix two drops of donor plasma and one drop of recipient red cell suspension for the minor crossmatch. Mix gently
10. For the recipient control, mix two drops of recipient plasma and one drop of recipient red cell suspension. Mix gently
11. Incubate the tubes at room temperature for 15 minutes
12. Centrifuge the tubes for 15 seconds at 1000g
13. Observe the plasma for haemolysis
14. Resuspend the centrifuged cells by shaking gently
15. Observe the red blood cells for agglutination

Interpretation: haemolysis or agglutination in a crossmatch indicates transfusion incompatibility. The degree of agglutination is graded 0 to 4+. If all available units of blood are incompatible, the least reactive should be used. When the recipient control shows haemolysis or agglutination, the crossmatch cannot be interpreted. This is common in patients with haemolytic anaemia.

Blood transfusion protocol

1. Select healthy donors. Vaccinated dogs over 20kg between 1-8 years on heartworm prevention, ideally DEA 1.1 negative. Cats over 4.5kg between 1-10 years that are negative for FIV and FeLV.
2. Sedate or anaesthetise the patient if required (recommended). We commonly use butorphanol 0.1-0.3mg/kg IV in dogs. Cats usually require deep sedation or general anaesthesia. We use 10mg ketamine and 0.5mg midazolam IV. Avoid acepromazine as it causes hypotension and platelet dysfunction.
3. Take the donor PCV/TPP and place on intravenous fluids, low rates until transfusion is taken then increase to replace 1.5-2 times the volume taken. Fluid replacement is not essential in healthy, conscious donors.
4. Aseptically prepare the site, almost always the jugular vein, the femoral artery has been used in some blood banks. Avoid venipuncture at the periphery of the prepared site, aim in the middle. LOOK AFTER the donor, avoid multiple sticks, apply pressure to a haematoma as soon as possible, use the other jugular if required, but keep the clipped site small. Apply Arenca cream if available. Reward the donor and avoid long lasting sedation.
5. Use anticoagulated collection bags with CPDA-1 for dogs, collect up to 10-20mls/kg, or 450ml blood to complete a unit. For cats use a 19G butterfly needle connected to a 20 or 60ml syringe with 1ml/7ml CPDA-1.
6. Administer the blood via any vein to the recipient (intraosseous route can be used). Start with a low rate, or a test volume of 1-2mls, then run the product at 0.25-2ml/kg/hr for the first 15 minutes, then increase the rate to 5-10ml/kg/hr. You can bolus the blood in severe hypovolaemia. Temperature, heart rate and respiratory rate should be monitored q10mins for 30mins, then q30mins from there on. The patient should be monitored for vomiting, diarrhoea, urticaria and haemoglobinaemia and haemoglobinuria. Any changes warrant a decrease or cessation of the transfusion.
7. The transfusion should be completed within 4 hours. Calculate the amount to give via the following equation: Use it as a rough guide, for haemolysis aim for 20%, for trauma aim for 35%. Very rarely do you reach your target accurately.

   Body weight (kg) x K x (desired PCV – recipient's PCV)/donor PCV
   (K = 88 for dogs and 66 for cats)

8. Post-transfusion PCV/TPP should be taken several hours after completion.

When using packed red blood cells, the refrigerated bag does not need to be warmed prior to use unless the patient is severely hypothermic or is a neonate. Warming decreases cell viability and promotes bacterial growth if contamination has occurred. If the transfusion needs to be given over 4 hours, or only half the contents are required, then a special separation bag system can be used.

Plasma needs to be thawed carefully prior to use, either at room temperature or in a luke warm water bath. It should be used within 4 hours of thawing.

Blood transfusion reactions

Transfusion reactions can occur with incompatible serum, platelets, white blood cells or erythrocytes. Alloantibodies to erythrocyte antigens will cause the most clinical severe reactions. There are four classes of transfusion reactions:

1. Acute immunologic

Acute haemolytic reaction
Febrile nonhaemolytic reaction
Urticaria

2. Acute nonimmunologic

Electrolyte disturbances
- Hypocalcaemia
- Hyperkalaemia
- Hypomagnesaemia

Embolism (air or clotted blood)
Endotoxic shock
Circulatory overload
Contamination of blood
Physical damage
- Freezing
- Overheating

Hypothermia
Dilutional coagulopathy

3. Delayed immunologic

Delayed haemolytic
Posttransfusion purpura

4. Delayed nonimmunologic

Infectious disease transmission
- FIV
- FIP
- FeLV

Haemochromatosis

Dr Anna Byron
Internal Medicine

THE 'NEW' COAGULATION CASCADE

HAEMOSTASIS

Haemostasis has two main aims under normal physiological conditions. The first is to keep blood circulating in a fluid, clot free state in normal healthy vessels. The second, and more obvious aim is to rapidly form a 'haemostatic plug' at the site of vascular injury.

Triggers of haemostasis in healthy individuals-

1. Vessel damage – Most commonly mechanical injury

Triggers of haemostasis in disease states-

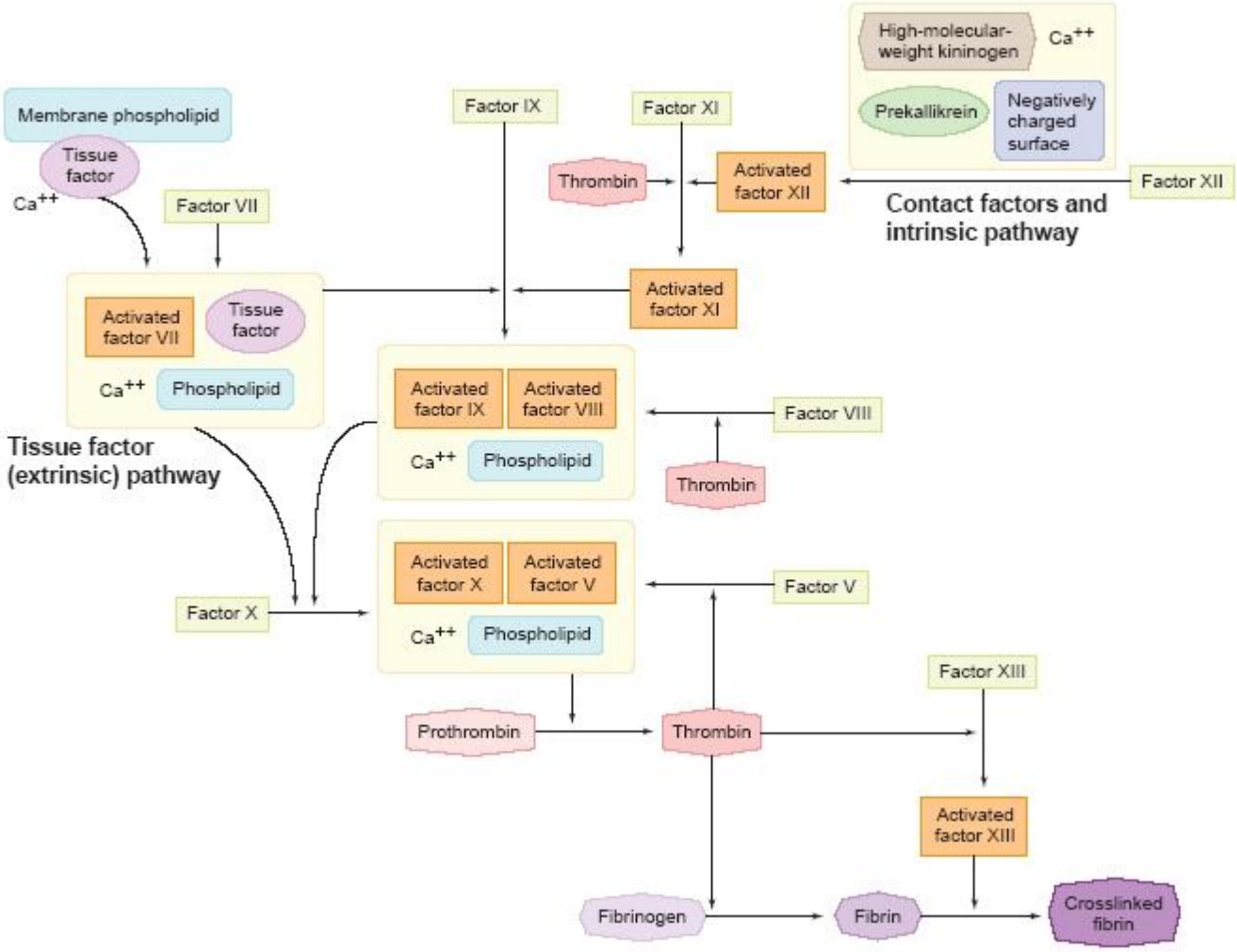
1. Bacterial or Viral injury to endothelial cells (eg vasculitis – more common than you think!)
2. Cellular processes
    a. Inflammation (eg pancreatitis)
    b. Cancer
3. Chemical injury to vessel walls

Vascular damage in both healthy and diseased states results in blood being exposed to tissues outside vessels, including sub-endothelial collagen. These tissues contain Tissue Factor (also called Factor III and thromboplastin), which initiates platelet aggregation and the 'coagulation cascade'. In healthy individuals, vascular damage is most commonly isolated to one location. In disease states, the damage is commonly more generalized, and coagulation occurs throughout the circulatory system, resulting in disseminated intravascular coagulation (DIC).

MODELS OF COAGULATION

To understand common coagulopathies and therapy, it is important to have a general understanding of the coagulation system. There are 2 main models of coagulation which are outlined below.

Cascade model



The Cascade model is a great model for **coagulation as it occurs *in vitro***. It does not take into account the role of cell surfaces, including platelets in coagulation, and assumes that all of these factors are available in circulation to be activated once the cascade starts. It also assumes that if the extrinsic pathway is altered or absent, that the intrinsic pathway will take over and vice versa. In reality, Haemophiliacs world over will tell you that if certain factors are not present, even if the other side of the pathway is intact that they will still bleed excessively if not completely. This model is best used to understand laboratory testing of coagulation disorders, including APTT and PT which will be discussed later.

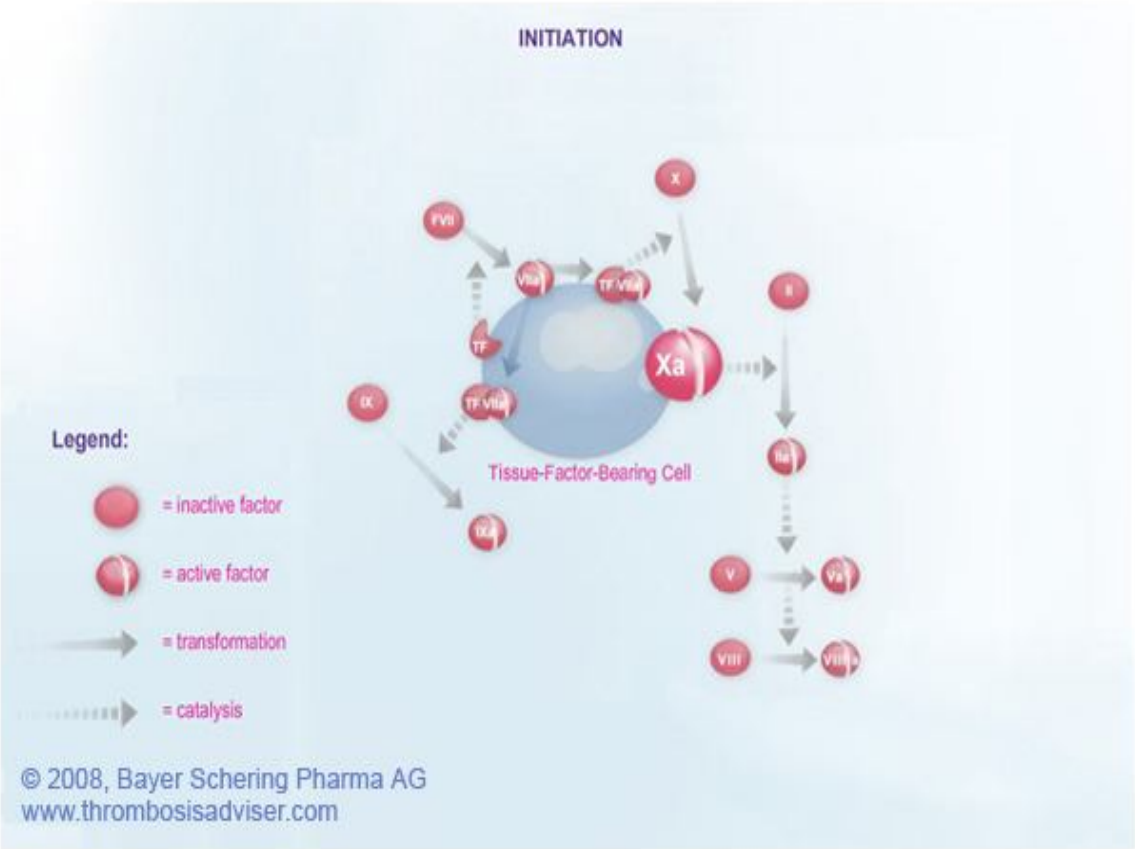
Cell based model – Key players: Tissue Factor, Factor Xa, Thrombin and Platelets.

This model takes into account the contribution of platelets and endothelial cells to coagulation. *In vivo,* clotting occurs to a small extent in the fluid state as the cascade model suggests, though the majority of thrombin formation occurs when the factors of the above model are bound to cell surfaces. This model divides coagulation into three phases;

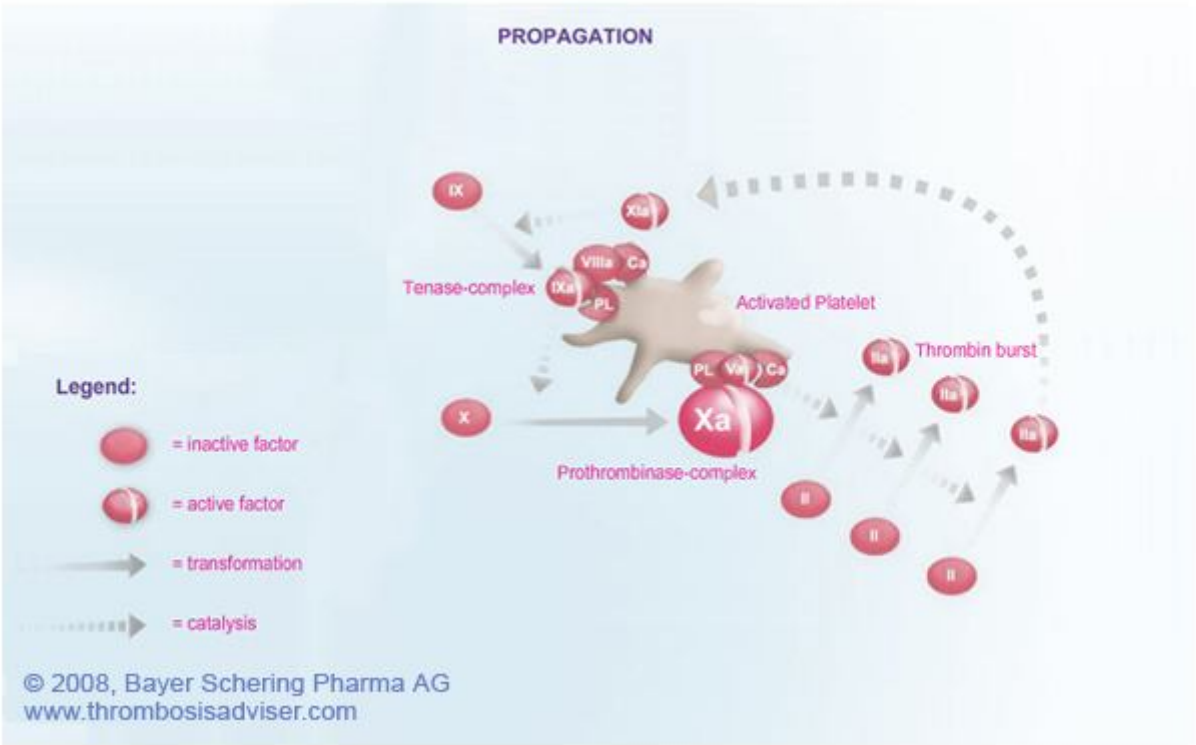
1. Initiation phase: The exposure of blood to Tissue Factor (so called because it exists in tissue, rather than the blood stream). The tissue factor **activates platelets** locally, and aggregates form a temporary clot at the site of the injury. The tissue factor interacts with factor VII to form factor Xa, which subsequently can produce small amounts of thrombin. You may notice that this is the **'extrinsic pathway'** of the above cascade model. The newly formed thrombin and activated Factor X (Xa) then

provide positive feedback, and tells the factors from the intrinsic pathway to set up for the propagation phase of coagulation. This is all occurring within the vessels.

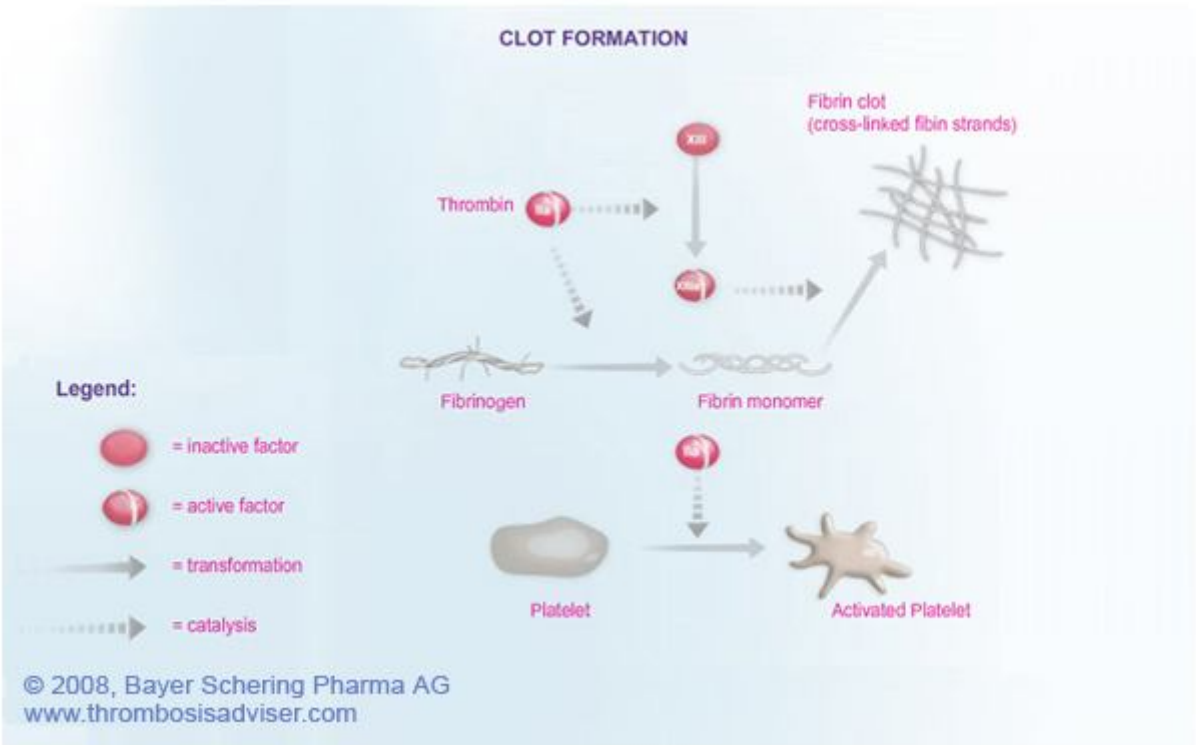
Don't get caught up in the numbers and interactions between factors, if you can remember the aim of coagulation is to produce thrombin, and the key players – Tissue factor and Factor Xa.



2. **Propagation phase**: There are 3 main parts to this phase
    a) **Platelets** that have been activated **externalize phosphatidylserine (PS)** on their surfaces. This is a phospholipid which is normally confined to the inner surface of cell membranes. Upon activation, PS is transferred to the external surface of the platelet membrane. PS acts as a scaffold for the factors of the intrinsic pathway to form complexes.
    b) There are 2 **complexes formed** – Tenase increases factor Xa generation, and Prothrombinase which converts prothrombin to thrombin. These complexes increase thrombin generation by 300,000 times compared with that produced in the initiation phase. PS may also be externalized in endothelial cells and monocytes at the site of vascular injury.
    c) The **Thrombin generated** has many roles- it converts fibrinogen to fibrin, but also has procoagulant roles by providing positive feedback by activating more platelets, and stimulating monocytes, fibroblasts, and smooth muscle cells.



3. **Clot formation phase:** The vast amounts of thrombin formed during the propagation phase convert fibrinogen to fibrin. Fibrin stabilizes the temporary clot formed by platelet aggregates. Once this fibrin is cross linked (by interaction with Factor XIII), only the fibrinolytic process will break it down.



ANTICOAGULANT MECHANISMS

Haemostasis is a balance between the phases of coagulation outlined above, and constant fibrinolysis. This balance is essential to prevent clots from flying around in circulation and causing embolic disease. There are 3 main proteins involved in inhibiting coagulation.

1. Antithrombin: Inhibits the effect of thrombin, factor X, IX and XI by binding and inactivating them. Heparin potentiates its inhibitory effects. This is most effective before factors become membrane bound, ie in the initiation phase of coagulation.
2. Activated Protein C: Thrombin activates this protein.which in turn deactivates the cofactors (Va & VIIIa) required for the amplification phase. In doing this, the amount of thrombin produced decreases – so thrombin also has a negative feedback loop.
3. Tissue factor pathway inhibitor: As the name implies, inhibits the Extrinsic or tissue factor pathway. This protein binds to factors VII and X, preventing them from contributing to the cascade.

In addition to these three proteins, endothelial cells are constantly oozing prostaglandins and nitrous oxide, which prevent platelet aggregates occurring in normal vessels.

Once a clot has formed, fibrinolysis occurs. This is a complex process of breaking down both soluble fibrin and cross linked fibrin. The key player in this process is **plasminogen**. Once broken down **fibrin degradation products** are produced, and are measurable in the blood. This can give an idea of the amount of clotting occurring, and has been used as an indication for DIC.

In healthy vessels, these **anticoagulant mechanisms predominate**. However, when vessel damage occurs for any of the reasons discussed above, coagulant proteins outweigh these mechanisms, and a clot is formed. When extensive clotting occurs, for example in extensive injury or DIC, this balance is lost, and clotting occurs without these tightly regulated controls.

COMMON COAGULOPATHIES

So how does this information apply to the disorders we actually see? Understanding the interactions of the different elements of the coagulation system can guide our testing and treatment.

RODENTICIDE TOXICITY

- Results in decreased functional activity of Factors II, VII, IX & X.
- The levels of these factors are normal, though cannot function normally as the warfarin or warfarin like rodenticides antagonize vitamin K.
- Vitamin K is required in synthesis of these factors – In the absence of Vitamin K, II, VII, IX & X can't be activated.
- Why does this effect coagulation?
    - No II = No thrombin = No fibrin = No clot.
    - No VII = No interaction with Tissue Factor = No 'extrinsic pathway' – or initiation phase of coagulation.

- Testing
    - Prothrombin Time (PT) is used to check the integrity of the 'extrinsic pathway'.
    - Activated partial Thromboplastin time (APTT) is used to check the integrity of the 'intrinsic pathway'.
    - In reality, APTT is just a really diluted PT. Dilution takes tissue factor out of the equation, therefore the APTT is measuring coagulation without TF contribution (ie much slower!!!).
    - The factors affected by rat bait are from both pathways, so both APTT and PT are elevated.

- Occasionally only the PT may be elevated – This is usually early on in the intoxication. Factor VII has a half life of 6 hours, much shorter than other factors, so viable VII runs out very soon after ingestion of rat bait. As VII interacts with TF, and TF is measured by PT only, it can be prolonged early on.
- These tests are performed on blood from citrate tubes. They should be run between 5 minutes and 2 hours after blood collection. Wiping with alcohol can prolong readings artificially, so use chlorhex or water if anything.
- Ideally, make sure the dog is calm during blood collection – Dog platelets are activated at the drop of a hat, which can alter the readings.

- Treatment
  - Supplement Vitamin K! We need to supplement at a level that exceeds the amount which can be antagonized by the ingested Rat bait.
  - If ingestion is not confirmed, a PT should be done 36 – 72 hours after exposure. The PT will be normal if you have started Vitamin K already, so don't stop the therapy based on this result.
  - Vitamin K dose – 3 - 5mg/kg bid
  - If Blood loss is life threatening, transfusion is indicated.

Type of Bait	**Minimum duration of therapy**
Warfarin	14 days
Bromodialone	21 days
Brodifacoum and others	30 days

THROMBOCYTOPAENIA

- Platelets are responsible for providing the primary plug when vessel damage occurs, while the rest of the cascade generates fibrin to stabilize it.
- Platelets also provide the scaffold (phosphatidylserine – PS) for the coagulation factors to cling to for the most propagation phase of thrombin generation.
- Therefore when platelet numbers are low 2 parts of the coagulation system are compromised.
- The lack of platelet plug formation results in petechiae and ecchymoses formation
- The decreased amount of thrombin produced means less fibrin is formed, and time to clot stabilization is drastically prolonged.
- If platelet numbers are <100,000/ul, measurable changes in thrombin generation occur. It is not until platelets are less than 40,000/ul that clinical signs of bleeding are observed.
- If platelet levels are at a level that abnormal bleeding is occurring, it is almost certainly due to immune mediated platelet destruction.

- Testing
  - Platelet estimate from **smear**: 1 platelet per 100x objective field = 20,000/ul. If the platelets are large this may indicate a left shift. If platelets are clumped it is impossible to estimate – Make sure your sample goes straight into either EDTA or Citrate tubes after drawing it.
  - **CBC** – Most machines do platelet counts.
  - **Buccal mucosal bleeding time**: Must be performed with standard equipment – very user dependent. If normal platelet number but BMBT prolonged, bleeding may be due to a thrombocytopathy.

- Treatment
  - Wrap in cotton wool. No jugular venepuncture, as there is not enough platelets to plug the hole!
  - Immunosuppress! Oral medications are preferable, but gentle administration! Soft palate haematomas get in the way of respiration.
  - Prednisolone 2-4mg/kg PO divided q12h
  - Cyclosporine 10-25 mg/kg/day PO divided q12h OR *Neoral*®: 5-10 mg/kg/day PO divided q12h. Measure a trough level 24 – 48 hours after starting if concerned about dose.

- Azathioprine 2mg/kg PO q 24h reducing to q48h after 1 – 2 weeks. Check CBC fortnightly and watch for signs of leucopoenia, pancreatitis and hepatotoxicity.
- Generally, platelet number increases do not occur until > 5 days after starting therapy. Managing complications and close monitoring is vital for this period of time.
- Rule out underlying disease such as neoplasia.

DISSEMINATED INTRAVASCULAR COAGULATION

- There is a lot of interplay between the inflammatory and coagulation pathways. So in reality, any disorder resulting in an intense inflammatory focus (eg pancreatitis), generalized systemic inflammation (eg IMHA), or extreme vascular damage (eg haemangiosarcoma or burns) can result in DIC.
- 2 phases:
  - Non-overt phase = subclinical, difficult to diagnose. During this phase thrombin formation and subsequently fibrin formation is occurring within circulation, not isolated to one focus of vessel damage. This is also sometimes called the hypercoagulable phase. There will be no evidence of bleeding, and more than likely no evidence of the numerous thrombi in circulation.
  - Overt Phase = consumption of coagulation factors and platelets to the point that the PT/APTT will be prolonged. These tests are only affected when factor levels get below 30% of normal. There is a high risk of bleeding during this phase.
- 50 % of dogs with IMHA in one study developed thromboembolic disease.

- Testing
  - No really reliable tests exist for DIC, particularly the non-overt phase.
  - **PT/APTT** are not prolonged until way too late to be useful.
  - **CBC** – The platelet number can help detect DIC. The platelet level does not drop dramatically in DIC, and may never fall below the reference range – though it will reduce significantly from the baseline level for a particular animal. A dog may have a platelet count of 500,000/ul when first hospitalized or on previous blood tests. If the count is 250,000/ul on next reading, it is still in the reference range, but there has been significant platelet consumption, and DIC may be occurring.
  - **D-dimers** – Only just available in Australia through human laboratories. D-dimers are a product of the degradation of cross linked fibrin, ie products of clot breakdown. If D-dimer levels are elevated, there is increased clot formation and breakdown occurring. This is the only reliable test for the detection of non-overt DIC.
    - Ask your local human hospital lab if they run the test – most are willing to help out. Most use Stago brand, latex agglutination technique. This is suitable for use with canine plasma, but other techniques aren't. The normal range is <0.28mg/L. Anything above this level is indicative of DIC. The test must be run within 2 hours of sample collection, using citrate tubes.

- Treatment
  - Removing the cause of systemic inflammation or vascular damage is the mainstay of halting progression. However this is rarely possible, and managing the conditions becomes more important.
  - Plasma transfusions provide clotting factors and anti-inflammatory effects – though some references say this is adding fuel to the fire. I generally use plasma in animals with acute inflammatory conditions such as pancreatitis.
  - Anticoagulant therapy – Indicated during the non-overt phase to prevent thrombus formation and further consumption of factors, though contra-indicated in overt phase. Realistically, there is no defined line between the 2 phases and both thrombus formation and bleeding are likely, so I generally use anticoagulant therapy regardless.
  - **Low molecular weight heparin** (*Fragmin*® *& Lovenox*®) decreases functional factor Xa by enhancing the effects of antithrombin. When Xa is low, thrombin generation cannot occur. This differs from unfractionated heparin in that it has much more predictable effects due to the degree of protein binding in circulation.

        - Fragmin®: 150u/kg SC q8hrs for dogs, 200u/kg SC q6hrs for cats. (there has not been a great deal of research in cats – 100u/kg q12h is not an anticoagulant dose, 250u/kg q6h is more than enough. I compromised here!)
    - **Clopidogrel** (*Plavix®)* decreases platelet aggregation, by blocking ADP receptors on the surface of platelets. This has been well studied and tolerated in cats to prevent thromboembolic disease secondary to cardiac disease, though not well defined in dogs. This drug targets platelets rather than inhibiting clotting factors, therefore targets multiple areas of the coagulation system.
        - Dogs: 3 – 5mg/kg PO q24h, Cats 18.75mg PO q24h.

VON WILLEBRANDS DISEASE

- von Willebrand's factor is a plasma protein (present in circulation) which acts as the Velcro to bind platelets to the vascular wall when vessel injury occurs.
- It works particularly well under situations of high sheer stress – eg arterial injury. It binds to subendothelial collagen then forms a complex with the platelets.
- There are 3 forms of von Willebrands disease in dogs
    - Type 1: Dobermans, Airedale, greyhound, wolfhounds, German shepherds and more are affected by this form. This is defined by having low concentrations of normally functioning vWF. This has variable severity clinically.
    - Type II: Pointers. Low concentration of vWF and inconsistent function. Moderate to severe form of disease.
    - Type III: Shelties, Scotties. vWF is completely absent, very severe.
- Type I is by far the most common. Dogs can be tested as carriers (heterozygous) or affected (homozygous). In Dobermans, clinical signs are seen when vWF concentrations are less than 15% of normal, though this is not consistent for other breeds.
- Reductions in vWF:Ag are generally seen in homozygous sufferers only.

- Testing
    - Most Dobermans from breeders have been tested as carriers or affected. 50% of the population are carriers!
    - **Buccal mucosal bleeding time** is prolonged in affected individuals with clinical pronation to bleeding. This is not diagnostic, but may indicate whether extra precaution should be taken during surgery.
    - Von Willebrand quantification is available through commercial labs.
    - If **vWF:Ag** 5-20%, Type I, If 1-5%, Type II, If <0.5%, Type III.

- Treatment
    - Cryoprecipitate supplementation during surgery for sufferers. Unfortunately this is not available in Oz.
    - FFP has very small amounts of vWF in it, though is better than nothing. Give at a rate of 10 -12ml/kg q12h
    - Whole fresh blood has moderate amounts of active vWF- Give at 12 – 20ml/kg q24h. This is limiting as fluid overload is common at rates greater than this.
    - Generally, pressure on a bleeding wound will facilitate haemostasis in mildly affected animals. This decreases sheer forces, enabling platelets to aggregate without vWF.