Vetnostics

Summer 2011/2012

Partners in Practice

- **Adrenals:** Parts 2: Clinical Signs
- **Feline herpesvirus ulcerative dermatitis**
- **KIT Immunohistochemistry for Canine Cutaneous Mast Cell Tumours**

Vetnostics

Welcome...

Welcome to the Summer 2011/2012 Vetnostics newsletter.

I hope you all had a festive end to 2011 and we look forward to another year of working with you as 'Partners in Practice'. In this current newsletter we continue the series on canine hyperadrenocorticism/hypoadrenocorticism along with updates on feline herpesvirus infection and canine mast cell tumours and various other bits and pieces.

As always, please contact me on 02 9005 7272 or by email (doug.hayward@vetnostics.com.au) if you have any requests/ideas for future newsletters or any other queries for that matter.

ADRENALS:
What you won't find in a textbook

Dr Sue Foster BVSc, MVetClinStud, FACVSc
Vetnostics Small Animal Medical Consultant

PART 2: CLINICAL SIGNS

Hyperadrenocorticism (hyperA)

1. Not every dog with hyperA will have polyuria (PU) and polydipsia (PD). PU and PD are common signs in hyperA but lack of PU/PD should not preclude investigation or diagnosis of hyperA. Similarly, and as a logical extrapolation, hyposthenuria and isosthenuria, whilst common in hyperA, are also not necessary for a diagnosis.

   PU is reportedly due to interference with ADH-mediated water resorption in the renal collecting ducts however, as it does not occur to any great extent in humans or cats (unless there is concomitant diabetes mellitus), then I suspect it may be due to a glucocorticoid-mediated "psychogenic" polydipsia in dogs. The fact that many dogs do not exhibit polydipsia when hospitalised and often concentrate their urine quite well in hospital adds further weight to this hypothesis.

   "Psychogenic polydipsia" in older dogs is nearly always due to hyperA and adrenal function testing should be performed prior to water deprivation testing in investigations of polydipsia in all patients other than those in which another cause is identified or congenital diabetes insipidus is suspected.

2. Up to 50% of dogs with hyperA are reported to have urinary tract infections (UTIs). HyperA should be considered in any older dogs with an UTI especially if the UTI is recurrent or relapsing.

3. Polyphagia is more consistent than polydipsia but is not necessarily evident in some dogs due to pre-existing ravenous feeding behaviour (eg Labradors, Beagles). Anorexia or inappetence in a dog suspected of having hyperA should prompt investigation of concurrent non-adrenal disease; non-adrenal disease may interfere with adrenal function test results.

4. Abdominal distension (pot-bellied appearance) occurs in >80% of cases though it may be subtle. It is due to redistribution of fat, muscle wasting and hepatomegaly. Urinary bladder over-distension may also contribute and occasionally in dogs, the over-distension results in bladder atony and dysfunction; this is reversible with catheterisation to relieve the bladder distension, and concurrent treatment of hyperA.

5. Intermittent abdominal bloating is a sign of hyperA that is often reported by owners and may even be the major presenting complaint. It is difficult to know whether this is due to polyphagia/over-eating, mild pancreatitis or some other cause. However, when "bloating" is due to hyperA, it resolves quickly with effective treatment.

6. Dogs with hyperA may have muscle wasting or decreased exercise tolerance. Decreased exercise tolerance and lethargy may not be noted as owners often attribute these to age and arthritis. Muscle wasting, especially temporal and paralumbar, tends to be more obvious in large breed dogs.
7. Skin and hair coat changes are well described but the following points are often not highlighted:
   - bilaterally symmetrical alopecia is rarely present in large breed dogs
   - lightening in coat colour and alteration in coat texture may be the only coat changes
   - solar bleaching can occur at the ends of the hair shafts because the hairs are not replaced as rapidly as normal
   - pyoderma may be the only sign of hyperadrenocorticism
   - excessive bruising (eg the bruising that occurs after careful venipuncture in that well behaved Maltese with the not-so-pleased owner) is common with hyperA
   - failure to regrow hair after clipping for venipuncture or surgery should prompt investigation of endocrine disease
   - resolution of chronic recurrent seasonal atopy or flea allergy dermatitis as a dog gets older, whilst great for the dog and owner, may be an indication of hyperA
   - calcinosis cutis is uncommon in spontaneous hyperA; the most severe cases of calcinosis cutis are usually iatrogenic.
8. Increased panting is reportedly due to increased fat deposition over the thorax and in the abdomen, wasting and weakness of the muscles involved in respiration and decreased pulmonary compliance. However, I wonder if panting is yet another "psychogenic" feature of hyperA in dogs as it does not occur in cats or humans and seems to happen very quickly after treatment with exogenous corticosteroids i.e. before any anatomical changes have had time to occur.
9. Always consider hyperA as a possible predisposing cause when older, not particularly active dogs present with cruciate ligament rupture (especially if their hair fails to regrow after surgery!).
10. Testicular atrophy in older male dogs may be due to hyperA. Failure to cycle and clitoral hypertrophy can occur in females: aged, intact, non-cycling females with hyperA are prime candidates for pyometra!
11. Neuromuscular signs are uncommon in hyperA. Myotonia is rare but if an aged dog presents with a very unusual gait (marked abduction of forelimbs, bizarre stumbling gait) and obvious muscle tone then think of this. Facial paralysis (unilateral or bilateral) is an exceedingly rare presenting sign. Neurologic signs of pituitary macroadenomas are often quoted but are quite rare; altered mentation, disorientation, ataxia and pacing are more common than seizures, coma and blindness.
12. Last, but not least, "old age/slowing down" is possibly the most common side effect of hyperA. Most owners note that their dog seems much younger once hyperA is successfully treated and many realise that they have incorrectly attributed "slowing down" to old age rather than hyperA for a long time (sometimes years).

Hypoadrenocorticism (hypoA)

1. Recurrent signs of gastrointestinal disease and a vague history of lethargy may be the only signs of hypoA, especially in dogs that have glucocorticoid deficiency only.
2. Cardiovascular collapse is not only due to mineralocorticoid deficiency and resultant hypovolaemia. Glucocorticoids are also important for cardiovascular function and cardiovascular collapse can occur in spontaneous and iatrogenic glucocorticoid deficiency.
3. Intermittent, difficult-to-localise abdominal or spinal pain responsive to prednisolone in a younger dog (especially if known breed predisposition e.g. Poodle or Fox Terrier) should prompt adrenal function testing for hypoA.

Feline herpesvirus ulcerative dermatitis

Dr David Taylor BVSc, Dip ACVP
Veterinary pathologist

This is an uncommon and typically facial disease caused by a herpesvirus that is indistinguishable from feline herpesvirus-1. The disease may be preceded by a bout of upper respiratory infection and/or cats may show chronic signs of sneezing and excess tearing. It is believed that stress and immunosuppression are important triggers of disease and may activate a latent infection in the trigeminal ganglia. Concurrent infections with FeLV and FIV are variably reported. Cats of any age are susceptible and there are no breed of sex related associations.

Grossly the lesions are characterized by facial erosions and ulceration with variable crusting. Predilection sites include the dorsal and lateral muzzle, nasal planum and periocular haired skin and lesions may be bilaterally symmetrical. Other less common sites for involvement include the distal extremities and the trunk.

Additionally, the lesions may be accompanied by regional lymphadenomegally, pruritus and may be painful. One in ten cats are reported to exhibit oral erosions and ulcerations.

The histopathological diagnosis is straightforward when the typical intranuclear inclusion bodies are evident. If inclusion bodies are absent PCR/ immunohistochemical staining may allow confirmation of the diagnosis.

Clinical differential diagnoses include facially predominant food allergy, mosquito bite hypersensitivity, pemphigus foliaceus and squamous cell carcinoma. It has been reported that cats with this disease that was initially misdiagnosed as a hypersensitivity reaction and subsequently treated with corticosteroids had exacerbation of lesions that assisted in ultimate and correct diagnosis.

Reference:

Skin Diseases of the Dog and Cat 2nd edition, 2005, by Gross, Ihrke, Walder and Affolter pg 124-126

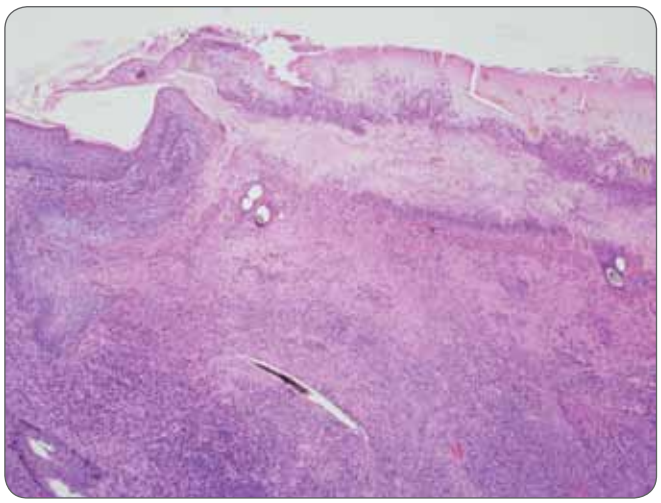


Figure 1: Histologic section of the skin characterized by epidermal ulceration, necrosis, inflammatory infiltrate and severe crusting.

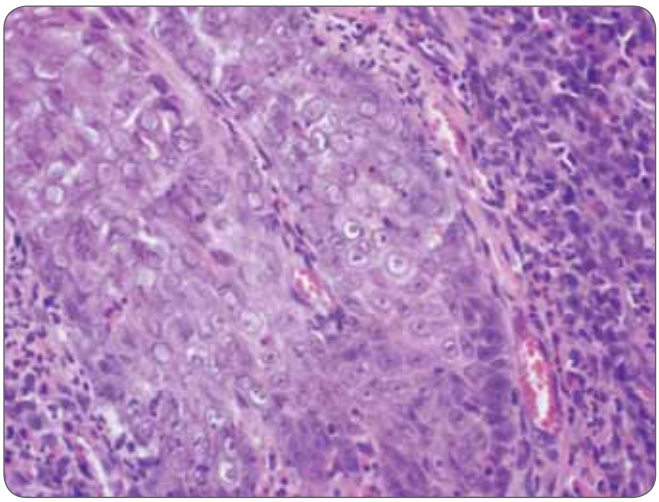


Figure 2: Higher magnification of the skin lesion showing intranuclear inclusion bodies within epithelial cells.

What is your diagnosis?

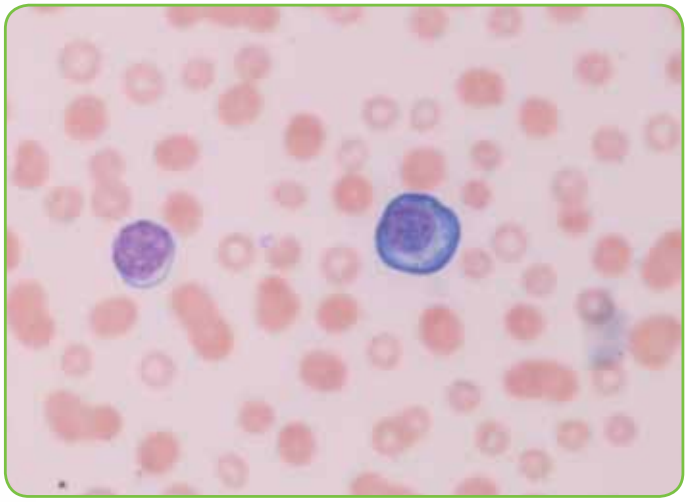


Figure 1: Wright's stained blood film, x100 (oil)

In this edition of 'What's Your Diagnosis?', we have an interesting case of rare atypical nucleated cells on blood film examination.

Please see the image on the left taken of an EDTA blood film from an 11y Cattle dog presenting with non-specific gastrointestinal signs and lethargy.

Apart from the unremarkable lymphocyte, what is the other nucleated cell and what is the possible significance of this finding?

Answer...

The further nucleated cell in the image is a plasmacytoid lymphocyte/plasma cell.

Plasma cells are present in lymphoid organs however are rarely identified in blood. Their presence in this case is very suggestive for the presence of underlying multiple myeloma given concurrent very marked hyperglobulinaemia identified (Figure 2 on the right demonstrates background staining and increased rouleaux associated with hyperglobulinaemia – same case as in Figure 1).

Plasma cells exhibit lower N:C ratios and increased cytoplasmic basophilia with respect to resting lymphocytes as well as possessing eccentric nuclei, perinuclear pale zone (Golgi apparatus) and coarse chromatin patterns.

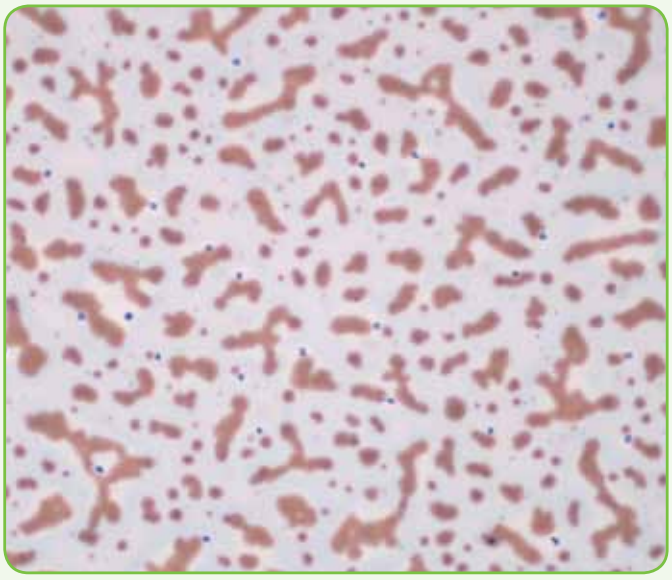
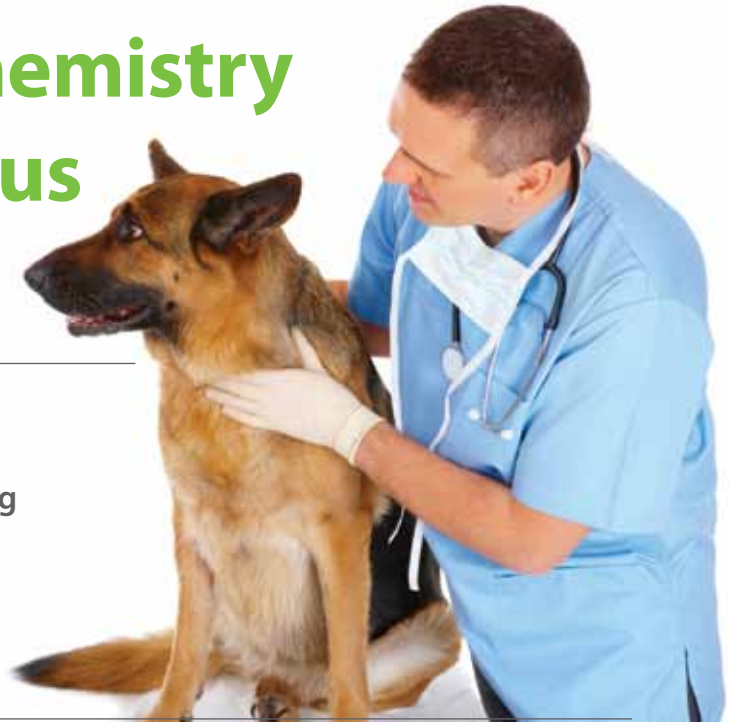


Figure 2: Wright's stained blood film, x20

KIT Immunohistochemistry for Canine Cutaneous Mast Cell Tumours



Mast cell tumours (MCTs) are one of the most common cutaneous neoplasms of the dog. They have highly variable biological behaviour, ranging from slowing growing nodules that are cured by simple surgical excision, to fast growing and invasive masses which rapidly progress to fatal widespread metastatic disease.

When a dog is diagnosed with a cutaneous MCT, clinicians often look to histopathological grading to clarify prognosis. However, wide variation in the outcome of dogs with grade two tumours, the predominance of grade two tumours, and variation among pathologist in designation of grade are commonly mentioned weaknesses of histological grading systems. We have recently revised our MCT reports to include the latest internationally recognised grading system (Kiupel) allowing MCT classification into low or high grade. It was found that application of the Kiupel grading system simplifies MCT categorisation, reduces variation in histologic classification and grades have prognostic significance. In addition, there has been considerable recent advancement in the understanding of the molecular basis of MCT development which appears to be promising for improving canine cutaneous MCT prognostication.

Up to 30% of dogs with cutaneous MCT have mutations of the c-KIT proto-oncogene which plays an important role in oncogenesis, especially for aggressive tumours. KIT, the protein product of c-KIT, is a receptor tyrosine kinase which normally functions to stimulate the cell cycle and is necessary for proliferation and differentiation of normal mast cells. Aberrant expression of KIT protein by neoplastic MCT cells has been shown to be a negative prognostic indicator in dogs.

Using KIT immunohistochemistry, three staining patterns can be observed in neoplastic mast cells. The first comprises perimembrane KIT staining, coinciding with the normal location of the protein in the cytoplasmic membrane of well-differentiated mast cells (Figure 1). Tumours with this KIT expression pattern are more likely to exhibit benign biologic behaviour.

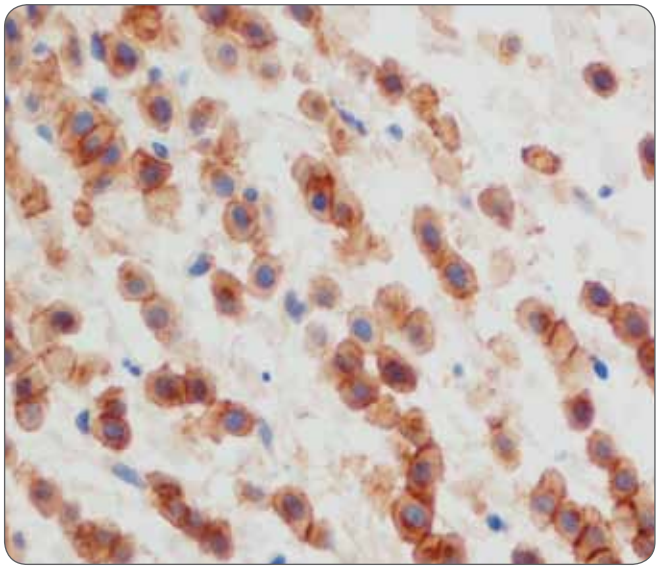


Figure 1.

The other staining patterns show increased expression of KIT in the cytoplasm (Figure 2), an abnormal location that suggests potential for downstream events which may lead to increased cellular proliferation. Dogs with tumours that have increased cytoplasmic KIT expression are at increased risk of poorer clinical

outcome, including increased incidence of local or distant recurrence, shorter disease free interval, and decreased survival time. When such patients have been treated with surgical excision only, more aggressive therapy may be consequently elected.

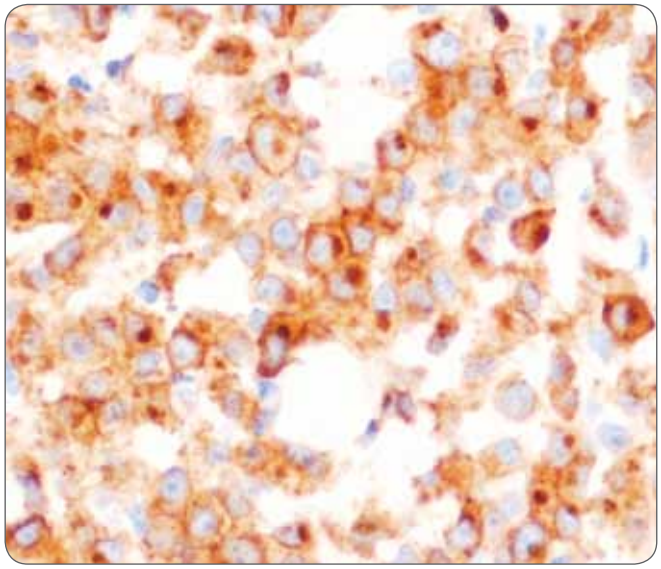


Figure 2.

Vetnostics has recently developed and validated a KIT immunohistochemistry test which reliably assesses the cellular localization of KIT, and it is available for $40.00 (+GST), with a turnaround time of 5 days. The test is performed on formalin-fixed paraffin-embedded sections from canine cutaneous MCT following routine histopathology. Presently, we recommend this test for grade two canine cutaneous MCTs to provide additional information, allowing for prompt identification of those dogs with tumours that may pose a high risk of aggressive behaviour, thus ensuring appropriate treatment strategies are utilised.

References:

Hahn, K.A., G. Ogilvie, T. Rusk, et al., Masitinib is safe and effective for the treatment of canine mast cell tumors. J Vet Intern Med, 22(6): p. 1301-1309, 2008.

Kiupel, M., J.D. Webster, J.B. Kaneene, et al., The use of KIT and Tryptase expression patterns as prognostic tools for canine cutaneous mast cell tumors. Vet Pathol, 41(4): 371-377, 2004.

Kiupel, M et al. Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. Vet Pathol. 48(1):147-55, 2011

London, C.A., P.B. Malpas, S.L. Wood-Follis, et al., Multi-center, placebo-controlled, double-blind, randomized study of oral toceranib phosphate (SU11654), a receptor tyrosine kinase inhibitor, for the treatment of dogs with recurrent (either local or distant) mast cell tumor following surgical excision. Clin Cancer Res, 15(11): p. 3856-3865, 2009.

Thompson, J.J., J.A. Yager, S.J. Best, et al., Canine subcutaneous mast cell tumors: Cellular proliferation and KIT expression as prognostic indices. Vet Pathol, 48(1): 169-181, 2011.

Webster, J.D., V. Yuzbasiyan-Gurkan, R.A. Miller, et al., Cellular proliferation in canine cutaneous mast cell tumors: associations with c-KIT and its role in prognostication. Vet Pathol, 44(3): p. 298-308, 2007.

Webster, J.D., V. Yuzbasiyan-Gurkan, D.H. Thamm, et al., Evaluation of prognostic markers for canine mast cell tumors treated with vinblastine and prednisone. BMC Vet Res, 4(32), 2008.

Sample handling for concurrent Cytology/ Histopathology submissions:

Although not a common occurrence, the concurrent submission of slides for cytology and fixed histopathology samples from the same animal is occasionally required (e.g. primary neoplastic mass for histology along with aspirates of the drainage lymph node or bone marrow cytology slides and a core biopsy).

In such cases, it is imperative that the cytology slides and histopathology samples are submitted separately – each having their own submission form and being 'double-bagged' as separate submissions. This prevents the deleterious effects of formalin fumes on the subsequent staining of cytology slides (this can be so severe as to render cytology submissions as non-diagnostic even if cellular and of otherwise good quality).

Vetnostics

Canine MDR1 Genotyping

Multidrug Resistance MDR1 gene expresses a protein involved in the transport of biological substances through the blood-brain barrier of dogs. A mutation (nt230[del4]) in the MDR1 gene has been shown to be predisposed in some breeds that can result in adverse reactions to some commonly administered drugs. In affected dogs, exposure of these drugs at the normal administered dose can result in serious neurological side effects such as hypersalivation, ataxia, blindness, tremor, respiratory distress and even death.

Vetnostics now offer Canine MDR1 genotyping which will classify a particular animal as **Normal, Carrier** or **Affected**. Sample required is a dry buccal (cheek) mucosa swab placed into a sterile container. The cost of this test is $65.00 excl GST. Please request under Other Tests section on the Vetnostics submission form.

Reference:
Klintzsch S., Meerkamp K., Doring B., Geyer J. (2010). Detection of the nt230[del4] MDR1 mutation in dogs by a fluorogenic 5` nuclease TaqMan allelic discrimination method. *The Veterinary Journal*. 185:272-277.

Hendra virus

As noted in various updates and in the media, Hendra virus-associated deaths in animals have been reported in specific areas of NSW and Queensland. Given the zoonotic potential of this virus and associated Health and Safety concerns in routine diagnostic laboratories, it is requested that Hendra virus is excluded (specific testing offered at EMAI and Queensland Government labs) where considered a clinical differential prior to submission of samples for further testing at Vetnostics. This will allow efficient processing of such samples without the requirement for phone calls, sample quarantine and subsequent sample forwarding for Hendra exclusion.

Many thanks.