Newsletter May 08

ML Pathology. | Vetnostics

Dr George Reppas
Specialist Veterinary Pathologist

Dr Sue Foster
Veterinary Medical Consultant

In this newsletter we have an article on Mast Cell grading. This has been adapted by George Reppas from a recent article by our colleagues at Vetpath.

Sue Foster has kindly updated and expanded a previous article on the use of the ACTH stimulation test in diagnosis and monitoring treatment of hyperadrenocorticism. This excellent article answers many of the questions we are frequently asked. If her advice is followed, considerable savings to your clients can occur.

Due to ever increasing costs we have felt it necessary to make some fee adjustments. These will have little if any impact on our regular users.

During the coming year we will be making announcements that we believe will further enhance our service provision and help us maintain our position as a leading provider of veterinary pathology in QLD and Northern NSW.

Activated clotting time tubes

Over the past couple of months, we have had numerous requests for the supply of activated clotting time tubes. Unfortunately the manufacturer has informed us that supply has been discontinued. We are presently investigating sourcing another supply of tubes for this test. As yet these tubes have not been validated for use in veterinary practice. They will also be considerably more expensive so free supply will no longer be possible. Availability of these will be notified through our web site.

Requesting tests not listed on our request form

These tests need to be requested in the space under OTHER TESTS and NOT as part of the history, otherwise our touch typists may not enter the information as a specific requested test and the test will not be carried out.

Discounts for vets and their staff

We believe that we offer very competitive discounts to veterinarians and their staff for their own pets. This will be maintained through 2008, we will have a 20% discount except on referral tests where a discount will not apply.

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UPDATE: Canine Mast Cell Tumours

Dr George Reppas

Specialist Veterinary Pathologist - *adapted from article by Vetpath*

Mast cell tumours (MCT) are one of the most common skin tumours in dogs and are lesions that are frequently encountered in clinical practice. These skin lesions have a wide variety of behaviours from small localised slowgrowing lesions with benign behaviour to multiple large and aggressive lesions with rapid growth and early metastasis to regional and distant sites.

A recent paper published in the Journal of Small Animal Practice (JSAP) has reviewed the recent literature and highlighted the advances in the diagnosis and management of cutaneous mast cell tumours.

Diagnosis of mast cell tumours is usually based on fine needle aspirate cytology or on histological assessment. Grading of tumours on histological examination is currently regarded as one of the primary determinants of the prognosis. However, the reliability of histological tumour grading alone has been questioned with significant inter-observer variation reported in recent studies.

Much of the grading is based on cell morphology, mitotic activity, as well as growth patterns of the tumour. The published grading parameters are a little vague with grey areas between grades, and few hard and fast criteria.

Much research effort has been directed at increasing the reliability and repeatability of the cutaneous mast cell grading system. Although the JSAP article discussed recent advances in predicting individual MCT behaviour (e.g. staining for argyrophilic nucleolar organising regions (agNORs) or using immunohistochemical stains to detect proliferating nuclear antigen (PCNA) and Ki67 antigen, as well as assessing KIT expression in MCT with c-kit mutations), these tests are not currently applicable to routine diagnostic pathology.

A very interesting recent development from UC Davis has been published in the May 2007 issue of Veterinary Pathology, which was not discussed in the JSAP paper. Their study uses the mitotic index (MI) as an indirect measure of the cell proliferation. MI is a simple measure of the number of mitotic figures in 10 standard high power fields. This is a simple and quick test to perform. In their study the MI was shown to be directly correlated to the tumour grade.

Furthermore the authors have shown that the median survival time for dogs with a MI of <5 was significantly longer (70 months) than for those with a MI >5 (2 months), regardless of tumour grade. Even in grade 3 MCT the dogs having tumours with a MI of <5 will have a long survival time compared to <2 months for dogs with a MI of >5.

It appears then that this relatively simple test can provide very valuable information at no extra cost to the client. The case numbers examined in this study were significant but not enough to dispel all doubt. More research is needed but may be slow to be published.

In future QML Pathology VETNOSTICS will endeavour to provide a MI as well as the tumour grade in all mast cell tumour cases submitted for histopathology.

REFERENCES:

1. Mitotic index is predictive for survival for canine cutaneous mast cell tumours. EM Romanski et al. Vet Pathol 44:335-341 (2007)
2. Cellular proliferation in canine cutaneous mast cell tumours: associations with c-Kit and its role in prognostication. JD Webster et al. Vet Pathol 44:298-308 (2007)
3. Advances in the diagnosis and management of cutaneous mast cell tumours in dogs. JM Dobson et al. Journal of Small Animal Practice 48,424-431 (2007)

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The ACTH Stimulation Test Revisited

Dr Sue Foster

Veterinary Medical Consultant

1. SCREENING TEST FOR HYPERADRENOCORTICISM

Advantages:

- only requires dog to be in clinic for 1h (compared to 8h for the LDDST)
- only requires two blood samples (not three as for the LDDST)
- detects iatrogenic hyperadrenocorticism
- less affected by disease stress than LDDST (better specificity).

This is the test of choice in an animal that has a reasonable chance of being concurrently affected by non-adrenal disease e.g. poorly controlled diabetes mellitus, renal disease, hepatic disease etc.

Disadvantages:

- the ACTH stimulation test is usually reported as being less sensitive than the LDDST for both pituitary dependent hyperadrenocorticism (up to 20% false negatives) and for adrenal tumours (up to 40% false negatives) although some authors have found similar sensitivities on both tests (Van Liew et al 1997). In the author's opinion, if dogs are carefully assessed for disease likelihood and appropriate cut-off points chosen for the ACTH stimulation test results, then this test appears to have good sensitivity.
- the test does not differentiate between pituitary dependent disease or that due to adrenal tumour(s).

2. MONITORING TEST FOR THERAPEUTIC EFFICACY

a) Mitotane: test needs to be run 36 to 48 hours after any dose of mitotane.

Mitotane interferes with cortisol synthesis in addition to causing adrenocorticolysis and as adrenal reserve is what is actually being monitored, direct mitotane effects on synthesis need to have worn off (otherwise adrenal reserve will appear less than it really is).

For good control of hyperadrenocorticism, both pre and post-stimulation results need to be <75 nmol/L. Poststimulation results 75-100 nmol/L tend to be associated with difficulty in achieving stable control though some dogs do remain stable at this level of adrenal reserve.

Post-stimulation results > 100 nmol/L are usually an early warning of loss of control if adequate induction results achieved. Clinical signs of loss of control are usually noticed by astute owners when post-stimulation cortisol concentration reaches 120-150 nmol/L. Less aware owners or owners of dogs that secrete less ACTH from their pituitary adenoma each day, may not notice loss of control until cortisol concentration reaches >200 nmol/L.

b) Trilostane: test needs to be run 4-6 hours after the morning-dose of trilostane to assess the peak effect of trilostane on cortisol synthesis. As trilostane does not usually last for 24 hours, an ACTH stimulation test 12-14 hour post-dosing may be needed to assess duration of effect.

Post-stimulation cortisol concentration with trilostane treatment should be 20-75 nmol/L.

Indications:

- screening test for spontaneous hyperadrenocorticism
- diagnosing iatrogenic hyperadrenocorticism
- monitoring efficacy of treatment with mitotane (Lysodren®) or trilostane
- diagnosing hypoadrenocorticism

QML Pathology Vetnostics Protocol:

- take a 0h blood into a serum tube
- inject 5µg/kg Synacthen® IV
- take another blood sample 1h later into a serum tube
- QML Pathology Vetnostics' post-stimulation reference range and thus interpretation is based on this protocol

Note: this dose has been validated in numerous studies (Watson et al 1998, Kerl et al 1999, Frank et al 2000, Behrend et al 2006, Martin et al 2007)

Other protocols:

- another frequently used protocol is to administer 250µg Synacthen® IV or IM with testing 1h later. This protocol is more expensive for the client. In addition, it may produce higher post-stimulation cortisol concentrations than the 5µg/kg IV protocol so if this protocol is used, you need to indicate both dose and timing on your submission form so that they can be taken into account when interpreting the result
- lower dose protocols with IV or IM Synacthen® (see below)

Older textbooks recommend that this test be performed in the morning. As dogs do not have a circadian rhythm for cortisol secretion, there is no scientific justification for this recommendation. This test can be run at any time. As fasted blood samples are preferable for chemiluminescence assays (radioimmunoassays are unaffected by haemolysis or lipaemia), this means that when performing an ACTH stimulation test in a diabetic dog with hyperadrenocorticism (e.g. usually fed before coming to the clinic), the test can be run late in the day with no problems.

Note: Synacthen ® is only registered for IM use in humans but does not seem to have caused problems in animals when given IV. A rare side effect, regardless of route administration, is acute adrenal necrosis!

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ABBREVIATIONS:

ACTH: adrenocorticotropic hormone

IM: intramuscular

IV: intravenous

LDDST: low dose dexamethasone suppression test

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1. Behrend EN, Kemppainen RJ, Bruyette DS et al. Intramuscular administration of a low dose of ACTH for ACTH stimulation testing in dogs. J Am Vet Med Assoc 2006:229:528-530
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3. Frank LA, Oliver JW. Comparison of serum cortisol concentrations in clinically normal dogs after administration of freshly reconstituted versus reconstituted and stored frozen tetracosactrin. J Am Vet Med Assoc 1998;212;1569-1571
4. Kerl ME, Peterson ME, Wallace MS et al. Evaluation of a low-dose synthetic adrenocorticotrophic hormone stimulation test in clinically normal dogs and dogs with naturally developing hyperadrenocorticism. J Am Vet Med Assoc 1999;214:1497-1501
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6. Van Liew, CH, Greco DS, Salman MD. Comparison of results of adrenocorticotropic hormone stimulation and low-dose dexamethasone suppression tests with necropsy findings in dogs: 81 cases (1985-1995). J Am Vet Med Assoc1997;211:322-325
7. Watson ADJ, Church, DB, Emslie DR, Foster SF. Plasma cortisol responses to three corticotrophic preparations in normal dogs. Aust Vet J 1998;76:255-257

TO REDUCE THE EXPENSE OF FREQUENT MONITORING

1) Reduce the amount of Synacthen® administered.

As Synacthen® is expensive, use a low dose ACTH protocol e.g. 5µg/kg IV for small dogs and 1 µg/kg IV for large dogs and store the remaining sample for future monitoring. Both doses have been proven to produce maximal cortisol secretion in healthy dogs (Martin et al 2007). If using really low doses, attention to timing of the post-stimulation sample is required. Timing needs to be PRECISELY one hour post injection for doses of 1 µg/kg or less (Martin et al 2007).

Intramuscular dosing with 5µg/kg has also been shown to cause maximal cortisol secretion (Behrend et al 2006). In the author's opinion, IV dosing is preferable unless patient difficulties preclude its use, as it ensures that the dose does reach circulation.

Only a small amount of Synacthen® is administered when using a 1- 5 µg/kg dose so **freeze any remaining sample**. Draw up the left-over Synacthen® in a 1 ml syringe (or draw up accurate doses into multiple 1 ml syringes), leaving a small air space at the end of the syringe. Cap each syringe, label it with dog name and date (frozen Synacthen® is stable for 6 months; Frank and Oliver 1998) and place it in the freezer. When that dog needs another ACTH stimulation test, **thaw** it, draw up the required dose and **re-freeze** the remainder. By doing this, multiple doses can be obtained out of one vial. This significantly decreases the cost of monitoring treatment: the owner can be billed for the whole vial initially but thereafter, until another vial is required, there is no more cost for Synacthen®, just fees for cortisol measurement and procedure.

2) Test only the post-stimulation cortisol concentration.

For monitoring efficacy, the important result is the poststimulation result as it indicates the extent of cortical destruction (mitotane) or decrease in cortisol synthesis (trilostane) or both (mitotane, if testing within 36 hours of dosing on suspicion of iatrogenic hypoadrenocorticism).

The first cortisol concentration really acts as a check on the endocrine assay. Whilst it is ideal to know the basal cortisol concentration as it can enable both practitioner and laboratory errors to be detected, it is often better for the client to be able to afford two good tests than one perfect test.