Corticosteroid-induced alkaline phosphatase (c-ALP)

Increased serum ALP is the most common routine laboratory abnormality in hyperA due mainly to the induction of a specific ALP isoenzyme by glucocorticoids. The corticosteroid-induced isoenzyme of ALP can be measured by electrophoretic separation, heat inactivation or more usually in commercial laboratories, by levamisole-inhibition. The levamisole inhibition explains why c-ALP is sometimes referred to as I-ALP but this terminology can be confusing as sometimes I-ALP is used to describe the liver isoenzyme; it is also referred to as CAP (corticosteroid-induced ALP) or SIAP (steroid-induced alkaline phosphatase). The sensitivity of c-ALP has been reported to be 0.81-0.95. The specificity is poor (0.18-0.41) and PPV in one study was as low as 21.4% thus this test cannot be recommended as a diagnostic test. Interestingly, only 50% of glucocorticoid-treated dogs had increased c-ALP in one study and that same study found that absence of c-ALP increase does not rule out spontaneous or iatrogenic hyperA (Solter et al 1993). Percentage c-ALP is often discussed. Wilson and Feldman found that c-ALP comprised 25% or greater of ALP in hyperA dogs and that hyperA could not be distinguished from exogenous glucocorticoid administration, liver disease or diabetes mellitus by percentage in this study. Similar to UCCr, c-ALP results cannot be used in rule in or rule out hyperA.

References:

PART 4b: UCCr and CALP

As part 4 of this series looks at diagnostic tests, we can’t escape some statistics. So, some very simplistic explanations relative to hyperA are as follows:

Sensitivity: the likelihood that the test will detect hyperA
Specificity: the chance that a positive test is truly hyperA

Then, there are predictive values which take into account the prevalence or likelihood of a disease in addition to sensitivity and specificity.

Positive predictive value (PPV): the chance of a positive result being indicative of hyperA in dogs with signs of hyperA (e.g. Can we confidently diagnose hyperA when we get a “positive” result?)

Negative predictive value (NPV): the likelihood that a negative result eliminates the possibility of hyperA in dogs with signs of hyperA (e.g. Can we rule it out with a negative result?)

Urine corticoid: creatinine ratio (UCCR)

It is commonly stated that this test is highly sensitive but poorly specific, that is, the test picks up most dogs with hyperA but is also positive in lots of other disease states. It is widely advocated as a screening test to RULE OUT hyperA but should we be doing that?

The literature confirms that the test has good sensitivity and poor specificity (as low as 21%). In addition, one study had a positive predictive value of only 3% and 7% (i.e. only 3% chance that a dog with increased UCCR actually had hyperA) so we certainly can’t use UCCR for diagnosis.

What about the negative predictive value: can we use the test as a “rule-out”?

NPVs range from 0.96 (Smiley and Peterson) to 0.99 (Soffner and Reusch) so it would seem that a normal UCCr should rule out hyperA. However, these papers were all written in the early to mid 1990s and not in Australia. In Jody Braddock’s Master’s Thesis (2002), the sensitivity of the UCCR was only 66%, as 13 of 38 animals with rigorously confirmed hyperA had a UCCR ≤15.

It is unclear whether the population in Braddock’s thesis is different to that in the other studies.

Percentage of dogs treated dogs had increased c-ALP in one study and that same study found that absence of c-ALP increase does not rule out spontaneous or iatrogenic hyperA (Solter et al 1993). Percentage c-ALP is often discussed. Wilson and Feldman found that c-ALP comprised 25% or greater of ALP in hyperA dogs and that hyperA could not be distinguished from exogenous glucocorticoid administration, liver disease or diabetes mellitus by percentage in this study. Similar to UCCr, c-ALP results cannot be used in rule in or rule out hyperA.
Hello again! In this final Vetnostics newsletter of 2012, we have the penultimate installment in the Cutaneous mycobacterial diseases in dogs and cats series – a source of useful and current information for when dealing with this increasingly common disease category. Additionally, we continue the series’ on Adrenal disease and frustrating histopathology cases/diagnoses.

We at Vetnostics hope you have a festive end to 2012 and look forward to an ongoing fruitful partnership (‘Partners in Practice’) with you in 2013 and beyond.

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

**Cutaneous mycobacterial disease in dogs and cats (Part 2):**

Dr George Reppas  
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Veterinary Pathologist

Dr Richard Malik  
DVSc, PhD, Dip Vet Anaesth, M.Vet.Clin.Stud, FACVSc (Feline Medicine) FASM  
Vetnostics Small Animal Medical Consultant

“...and although adjacent structures such as the abdominal wall can be affected eventually, spread to internal organs or lymph nodes is very unusual. Even cats with extensive cutaneous lesions may have few signs of systemic illness. Such affected cats may develop constitutional signs of malaise, pyrexia, inappetance, weight loss, and reluctance to move. Occasionally, cats develop hypercalcaemia of granulomatous disease, although this is rarely, if ever, symptomatic.

**Canine mycobacterial panniculitis:**

There are substantially fewer reports of RGM infections of the skin and subcutis in dogs where the majority of cases represent localized infections in immunocompetent individuals. There is a trend in recent years for infection of the subcutis and skin with any RGM. The other 2 syndromes which can be involved in RGM disease include pyogranulomatous pneumonia, and disseminated systemic disease.

**Feline mycobacterial panniculitis:**

Panniculitis is the most common clinical presentation of infection with RGM. Numerous solitary feline case reports and smaller case series have been reported in the veterinary literature. In Australia, organisms from the M. smegmatis group are able to overcome the normal host defenses and establish infection in these “fatty” subcutaneous tissue regions (e.g. subcutaneous panniculus and especially the inguinal fat pad of cats) - particularly in obese individuals (often desexed females) in which there is a greater tendency for this disease to occur. In cats, infection often tends to start in the inguinal region (although sometimes it can begin in the axillae, flanks and dorsum). The disease may subsequently spread to contiguous areas of the lateral and ventral abdominal wall, perineum and tail base.

Mycobacterial panniculitis in cats initially tends to present with a circumscribed plaque or nodule of the skin and subcutis at the site of injury, although trauma is not reported in every case. Often the initial clinical suspicion is for a catfight abscess, although the absence of the foetid odour and turbid pus should alert the clinician to the possibility of an unusual infectious aetiology. Indeed, many such lesions treated with surgical drainage and antibiotics effective against obligate anaerobes are followed by wound breakdown and development of a non-healing suppurating tract surrounded by indurated granulation tissue. Later in the clinical course the subcutaneous tissue becomes thickened and the overlying skin becomes adherent, alopecic and punctuated with fistulae (Figure 1), which discharge a watery exudate (the so called “pepper pot” appearance). Thin areas of epidermis overlying subcutaneous collections of pus lead to characteristic focal purple depressions intermingled with the fistulae. Over time, the depth and breadth of the infected area increases and may eventually involve the entire ventral abdomen, adjacent flanks and limbs.

**Algorithm 1 - Overview of cutaneous mycobacterial infections with particular emphasis on cutaneous RGM infections in dogs and cats**

CUTANEOUS MYCOBACTERIAL DISEASE IN DOGS AND CATS (PART 2):

**Introduction:**

Here we continue the discussion on ancillary laboratory testing currently used to diagnose the various manifestations of cutaneous mycobacterial infections in dogs and cats and the therapeutic modalities currently available for their treatment. In this article we focus upon cutaneous mycobacterial infections in dogs and cats caused by rapidly growing mycobacteria (RGM) (Algorithm 1).

**Cutaneous mycobacterial infection in dogs & cats**

**Rapidly growing mycobacteria**

- **Non-cultivable or poorly cultivable mycobacteria**
- **Slow growing mycobacteria**
  - **M. bovis**
  - **M. microti**

**Mycobacterial (infectious) panniculitis**

**Refer to previous Vetnostics article**

**Refer to previous Vetnostics article**

**Figure 1:** Typical clinical presentation of a cat with mycobacterial panniculitis

**Figure 2:** Dog with mycobacterial panniculitis – lesion as it appears in situ and after excisional biopsy.

**Diagnosis of feline and canine mycobacterial panniculitis:**

Diagnosis of cutaneous RGM infection is relatively uncomplicated, provided the clinician has an adequate index of suspicion and the laboratory has been informed that a mycobacterial infection is suspected so that special procedures can be employed. Diagnostic material can be collected via fine-needle aspiration or biopsy. Cytologic and histologic preparations can be stained to look for acid fast bacilli (AFB). PCR testing can also be performed on cytological specimens as well as histopathology specimens, although it is important that clean slides are used as mycobacteria can be present in dust. Pus and tissue homogenates can be cultured using routine mycobacterial media.
CUTANEOUS MYCOBACTERIAL DISEASE IN DOGS AND CATS (PART 2) continued...

**Specimen Collection and Submission from skin and soft tissue infections:**
In our experience, samples of pus obtained from aspirates of affected tissues through intact skin provide the best specimens. This material can be obtained from a palpably abnormal portion of the subcutis. The overlying skin should be disinfected with 70% ethanol prior to obtaining material. The ROMANOWSKY-stained cytology slide (but not other stains) using a generic *Mycobacterium* PCR (MYCPKR) assay to identify the RGM. Attesting culture of the lesion in question once a cytological diagnosis or suspicion of RGM has been raised is preferred (see below).

**Organism Cultivation and Antimicrobial Susceptibility Testing:**
There is great value in obtaining RGM species identity and susceptibility data in every case, as this has a big impact on the culture and antibiotic resistance data. Susceptibility testing of RGM is not only useful for clinical purposes, but historically has also been used to provide phenotypic data for typing of isolates (for example, *Mycobacterium fortuitum, M. chelonae, and M. abscessus*). This information can be gained from a mycobacterium reference laboratory following phenotypic antibiotic susceptibility to trimethoprim, polymyxin B, and imipenem. This data is different in different regions.

Other practicalities, the choice is generally reduced to one or a combination of a fluoroquinolone, doxycycline, or clarithromycin. Amikacin is superior to gentamicin, although it is substantially more expensive in Australia. Another option would be moxifloxacin (10mg/kg slowly IV once daily), as it is available as a parenteral formulation.

**Cytopathologic Findings:**
Histopathological lesions are typically characterized by pyogranulomatous inflammation. The fluid aspirates of oral or acute fast bacilli (AFB) are visible using appropriate stains such as modified ZN. AFB are most often found in lipid vacuoles (Figure 5).

**Pathologic Findings:**
Histopathologic lesions are typically characterized by pyogranulomatous inflammation. The fluid aspirates of oral or acute fast bacilli (AFB) are visible using appropriate stains such as modified ZN. AFB are most often found in lipid vacuoles (Figure 5).

**Therapy:**
Although the medical and surgical management of mycobacterial disease is well described the introduction of new antimicrobial agents such as the fourth generation fluoroquinolones, moxifloxacin and prazidoxime may be appropriate agents (for example, the *C. teglicycine*) means that therapeutic recommendations continue to evolve (R. Malik unpublished observations).

Historically, these infections were considered very difficult to treat and to a large extent, treatment regimens are based on the extent of the lesion as a reason for the chronicity, severity, and refractoriness of these infections. The more recent use of intrapleural antimicrobial agents directed at high pressure refractory to treatment, particularly those caused by *M. fortuitum* and *M. abscessus*, which are more common in the United States than in Australia.

Briefly, treatment should commence with one or two oral antimicrobials (doxycline, a fluoroquinolone, and/or clarithromycin) initially chosen empirically (considering local knowledge of the mycobacterial species) and then subjected to susceptibility testing in vitro. Dosages for these drugs are listed in Table 1. Long-term administration of such an agent or agents is sometimes sufficient to achieve a cure over several months, but in more severe cases, surgical resection of recalcitrant tissue is eventually necessary. Given the extent and severity of the pathology in many of the species, understudied, adequate levels of antimicrobials may not be achieved throughout all affected tissues. In such instances, the best chance for a successful outcome is to remove as much infected tissue as possible following preliminary antimicrobial therapy. *Residual foci of infection can then be targeted by the high concentrations of antibiotics resulting and after surgery.*

Preni- and postoperative antimicrobial therapy is vital to ensure primary intention healing of the surgical incision. Specific Recommendations

Once a tentative diagnosis of mycobacterial panniculitis is made, it is highly positive. Because a primary culture takes 3 to 4 days, with an additional similar period required for susceptibility testing, the initial choice of one or more antimicrobials must be guided by retrospectively acquired microbiology data. This data is different in different regions. *M. smegmatis* group isolates (most common in Australia) are susceptible to a wide range of antimicrobial agents suitable for treating chronic infections, except clarithromycin to which a majority of strains show inherent resistance. In contrast, *M. fortuitum* group isolates (the majority of RGM agents) and other species often have higher MICs for agents to which strains are susceptible. Thus, MICs of *M. chelonae*-abscessus group isolates tend to be lower than those of the *M. smegmatis* group, and intermediate from clarithromycin and linezolid. *M. fortuitum*, *M. chelonae*-abscessus group isolates, and *M. abscessus* are susceptible to amikacin, imipenem, and linezolid. The most effective combination of a fluoroquinolone, doxycycline, or clarithromycin for mycobacterial panniculitis is well described (R. Malik unpublished observations).

Once susceptibility data becomes available, the optimal drug or drug combination should commence using standard dose rates. Subsequently, the dose is increased slowly (over several weeks) until adverse side effects develop (if any). To reduce the need for surgery or dose reduction or until a convincing clinical improvement is observed.

Some animals treated in a preliminary fashion using orally administered antimicrobials may improve to such an extent that surgery becomes unnecessary. These animals can be cured using medical therapy alone, although treatment with oral antimicrobials in cats and dogs can be quite protracted. Surgery is required to resolve without the need for further surgical intervention involving a lesser depth of tissues than those that require surgery. Some lesions are not well perfused, and considerable diffusion barriers prevent blood levels of some antibiotics in organisms in deep subcutaneous tissues. Treatment should commence using standard dose rates. Subsequently, the dose is increased slowly (over several weeks) until adverse side effects develop (if any). To reduce the need for further surgical intervention involving a lesser depth of tissues than those that require surgery. Some lesions are not well perfused, and considerable diffusion barriers prevent blood levels of some antibiotics in organisms in deep subcutaneous tissues. Treatment should commence using standard dose rates. Subsequently, the dose is increased slowly (over several weeks) until adverse side effects develop (if any). To reduce the need for further surgical intervention involving a lesser depth of tissues than those that require surgery. Some lesions are not well perfused, and considerable diffusion barriers prevent blood levels of some antibiotics in organisms in deep subcutaneous tissues. The critical surgical consideration is to remove as much abnormal subcutaneous tissue as possible, which in some animals may necessitate the removal of very large portions of infected tissue. The overlying skin should be disinfected with 70% ethanol prior to obtaining material. The ROMANOWSKY-stained cytology slide (but not other stains) using a generic *Mycobacterium* PCR (MYCPKR) assay to identify the RGM. Attesting culture of the lesion in question once a cytological diagnosis or suspicion of RGM has been raised is preferred (see below).
Of the agents suitable for post-operative therapy, the fluoroquinolones (e.g., marbofloxacin, orbifloxacin, and moxifloxacin) are the drugs of choice. However, they should not be given systemically after 30 days because of their potential to cause retinal toxicity. Other tetracyclines should not be used post-operatively because they are less effective and can cause more side effects. For this reason, doxycycline should be given only immediately before or after surgery, or, if used as preventive therapy, at least 30 minutes before surgery.

The duration of therapy should be at least 6 weeks. If there is no clinical improvement, the treatment should be continued for at least 12 weeks. If the infection is not resolved by 12 weeks, the antibiotics should be changed to a different class of drug.

It is important to remember that the infection may persist for many months after the antibiotic therapy has been stopped. Therefore, the discontinuation of therapy should be based on clinical improvement, not just on laboratory findings.

The most difficult and frustrating histopathological diagnosis

**Dr David Taylor** BVSC, Dip ACVCP Veterinary pathologist

**Conclusion:**

Mycobacterial panniculitis is an eminently treatable disease. Diagnosis is straightforward, especially for practitioners familiar with the syndrome. The prognosis is good, even in cases with severe, extensive, and longstanding disease. Treatment involves long courses of antimicrobials chosen on the basis of laboratory testing sometimes combined with extensive surgical debri ment and wound reconstruction. Furthermore, the routine prophylactic use of antibiotics following treatment of penetrating injuries in obese dogs and cats may prevent the development of these deep-seated infections.

Finally, some centres are evaluating vacuum-assisted wound closure technology, which is in very early stages and may prove to be effective in obese patients with a high risk of infection. Vacuum-assisted closure technology is not suitable for dogs with a high risk of infection.

**References:**


**Table 1: Drug Dosages for Treatment of Rapidly Growing Mycobacterial Infections**

<table>
<thead>
<tr>
<th>DRUG</th>
<th>SPECIES</th>
<th>DOSE</th>
<th>ROUTE</th>
<th>INTERVAL (HOURS)</th>
<th>DURATION (WEEKS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>B</td>
<td>2 mg/kg</td>
<td>SC, IM</td>
<td>8–12</td>
<td>2–4</td>
</tr>
<tr>
<td>Amikacin</td>
<td>B</td>
<td>5–10 mg/kg</td>
<td>SC, IM</td>
<td>8–12</td>
<td>2–4</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>B</td>
<td>5–10 mg/kg</td>
<td>PO</td>
<td>12</td>
<td>5–12</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>B</td>
<td>15–30 mg/kg</td>
<td>PO</td>
<td>12</td>
<td>4–6</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>B</td>
<td>5–10 mg/kg</td>
<td>PO</td>
<td>24</td>
<td>12–52</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>B</td>
<td>10–20 mg/kg</td>
<td>PO</td>
<td>12</td>
<td>12–52</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>B</td>
<td>10–20 mg/kg</td>
<td>PO</td>
<td>12</td>
<td>12–52</td>
</tr>
<tr>
<td>Clavulanic acid</td>
<td>B</td>
<td>5 mg/kg</td>
<td>PO</td>
<td>24</td>
<td>12–52</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>B</td>
<td>8–12 mg/kg</td>
<td>PO</td>
<td>24</td>
<td>12–52</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>B</td>
<td>10–15 mg/kg</td>
<td>PO</td>
<td>12</td>
<td>12–52</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>B</td>
<td>3.5 mg total</td>
<td>PO</td>
<td>12–24</td>
<td>12–52</td>
</tr>
</tbody>
</table>

**8.** Both dog and cat, B, cat; D, dog; SC, subcutaneous; IM, intramuscular; PO, by mouth.

**a** For specific information about each drug consult Drug Formulary. Appendix II.

**b** Dose per administration at specified interval.

**c** Monitor blood urea nitrogen weekly for evidence of nephrotoxicity; often combined with other drugs. Cannot use long term.

**d** Use monohydrolated salt, if available; to minimize oesophageal irritation given before or with food, or followed by a small amount of water.

**e** Must check haemogram weekly for evidence of myelosuppression. Cannot use long term.

**f** For the dosage of other quinolones, see Drug Formulary.

**g** Avoid in young animals.

**h** Avoid higher doses or parenteral use in cats because of risk of retinal toxicity.

**i** Because of difficulty of fractionating liquid in capsules, cats are usually given one 10 mg capsule per dose. The contents of capsule may be cut in halves with a scalpel blade while wearing disposable gloves and dividing it into two gelatin capsules. Alternatively, a compounding pharmacist can provide the optimal dosage size.

**The most difficult and frustrating histopathological diagnosis**

**When these guidelines are not followed a mismatch between your expectations and pathologic findings can result. The decision to biopsy an animal on the basis of a single observation of elevated enzymes is a good example. Take the following scenario: A dog ingests a hepatic toxin and liver injury develops in 24–48hrs. The dog is ill and taken to your office and you learn that myeloid-related enzymes are increased, so you schedule a biopsy the next day. Given the half-life of ALT (2.5 days) the dog may have significantly elevated enzymes three days after the injury, but the hepatic repair process can clear the necrotic hepatocytes and replicate hepatocytes to replace the lost cells yielding a normal looking liver. You point out that ALT is 1000 U/L, yet I say the liver is normal and you suggest that not all Board certified pathologists are competent.**

**Finally, proper handling of the biopsy at the time of collection is important. Avoid excessive force to collect the sample so they can introduce significant crush artifact. Rapid formalin fixation will avoid drying and early degeneration.**

**Next Newsletter… Inflammatory bowel disease**

**Abnormal serum enzymes and function tests for 30 days or more**

**Hepatomegaly/microhepatica of undetermined cause**

**Hepatic involvement in systemic disease**

**Staging of neoplastic disease**

**Evaluation of response to therapy or progression of disease**