

Laboratory updates: Acute phase protein tests for diagnosis and monitoring of disease in small animals

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In the following paper Rory Bell and David Eckersall give a clinical introduction to the diagnostic use of Acute Phase Protein analysis in small animal medicine. David Eckersall is the leading expert in this field in the world and has presented his research findings widely over the last few years. This included a series of lectures at the American College of Veterinary Pathology meeting in 2006, which was reported in summary in this column (March 2007). The following review is timely and relevant because these tests are now increasingly commercially available and have a significant impact on diagnosis and case management.

INTRODUCTION

The acute phase proteins (APP) are blood proteins, the concentration of which increases in response to infectious and inflammatory disease being stimulated by cytokines such as tumour necrosis factor and interleukins 1 and 6. Their concentration in serum or plasma increases by at least 25% and can be increased by 1000 fold the level in healthy animals. Measuring the concentration of the APP can provide valuable information for diagnosis and prognosis of disease, for monitoring responses to therapy, and for general health screening (Ceron et al. 2005; Paltrinieri 2007a ;Paltrinieri 2007b).

Acute phase proteins are classed as being 'major', 'moderate', 'minor' or 'negative' depending on the relative changes in their circulating concentrations following an inflammatory insult (Fig. 1). The major APPs tend to have the most dramatic, but also short-lived elevations in response to inflammatory disease. Moderate and minor APPs have smaller, but longer lived elevations in circulating concentrations. The major, moderate and minor APPs are different between cats and dogs (Table 1). In both species, albumin is the most important negative APP; concentrations of albumin will slightly decrease in inflammatory disease, largely due to a shift in hepatic production to positive acute phase proteins in favour of albumin.

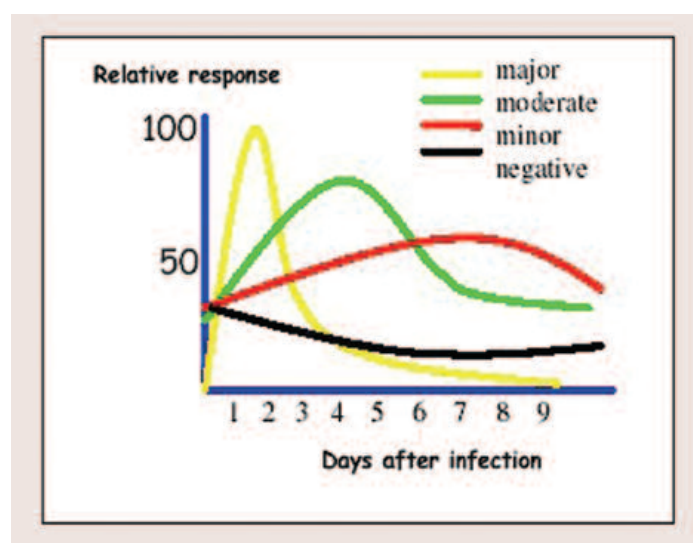


Fig. 1: Relative responses of APPs following stimulation.

TABLE 1: Major moderate and minor APPs in dogs and cats

Species	Major APP	Moderate/minor APP	Negative APP
Dog	C-reactive protein Serum amyloid A	α_1 -acid glycoprotein Ceruloplasmin Haptoglobin	Albumin
Cat	Serum amyloid A α_1 -acid glycoprotein	Haptoglobin	Albumin

APPLICATION IN CANINE MEDICINE

In dogs, C-reactive protein (CRP) and serum amyloid A (SAA) have low concentrations, (< 1 μ g/L) in the serum of healthy animals but the concentration increases over 1000 fold on stimulation reaching a peak 24-48 hours after the insult and falling rapidly during recovery (Fig. 1). Haptoglobin (Hp) and α_1 -acid glycoprotein (AGP) are moderate APP showing an increase of 2-5 fold on stimulation though Hp is also affected by corticosteroid (see below).

CPD

In the dog, CRP is a major APP with its serum concentration increasing rapidly from < 5 mg/litre to over 100 mg/litre. Recent research at the University of Glasgow and elsewhere has shown that the serum concentration of CRP can increase rapidly in dogs with arthritis, lymphoma, inflammatory bowel disease (Jergens *et al.*, 2003), in haematological diseases (Tecles *et al.*, 2005) and in many infectious diseases, such that there is a growing body of evidence on the value of these tests in a wide range of conditions (Ceron *et al.*, 2005; Paltrinieri 2007b). A list of disease conditions that can affect CRP concentrations in dogs is presented in Table 2. This list is unlikely to be exhaustive; its almost certain that further research will add considerably to the number of disease conditions known to affect CRP concentrations in dogs.

TABLE 2: Canine diseases where an acute phase response has been described (Ceron *et al.* 2005).

Acute Phase Protein	Disease/condition
CRP	Surgical trauma Rheumatoid arthritis Polyarthritis Intestinal obstruction Inflammatory bowel disease Lymphoma Acute pancreatitis Pyometra Pneumonia E. coli endotoxaemia Babesiosis <i>Bordetella bronchiseptica</i> <i>Ehrlichia canis</i> Leishmaniasis Leptospirosis Parvovirus Trypanosomiasis Bacterial enteritis
Haptoglobin	Surgical trauma Leishmaniasis Trypanosomiasis Cushing's syndrome Corticosteroid treatment
SAA	Parvovirus <i>Bordetella bronchiseptica</i> Leishmaniasis
AGP	Parvovirus Babesiosis <i>Ehrlichia canis</i> Lymphoma Carcinoma Sarcoma

Haptoglobin, in terms of its response to inflammatory/infectious disease, is classed as a moderate APP in dogs. Canine Hp is however particularly sensitive to corticosteroid and elevated levels of Hp are found both after treatment with corticosteroids and during naturally occurring hyperadrenocorticism (Harvey and West 1987; McGrotty *et al.*, 2005; Martinez-Subiela *et al.*, 2004). This is a potential disadvantage in monitoring inflammatory disease with canine Hp as steroid

treatment could interfere with interpretation, but full understanding of this process may reveal novel uses for the Hp assay (see below).

In the dog the circulating concentration of SAA does increase during an acute phase response and has been observed in experimental parvovirus infection (Yule *et al.* 1997) and in leishmaniasis (Martinez-Subiela *et al.* 2002). However with CRP being the major canine APP it is likely that SAA, a moderate APP in dogs, will be used in a secondary role in monitoring the acute phase response in this species.

APPLICATIONS IN FELINE MEDICINE

In the cat, SAA and AGP are the major APPs, and Hp is a minor APP. Although there have been relatively few investigations of the feline acute phase response (Paltrinieri 2007a), several conditions have been associated with raised levels of these blood proteins (Table 3). In addition, the measurement of AGP in feline serum and peritoneal fluid has become a recognised differential test for the identification of feline infectious peritonitis (FIP) (Duthie *et al.* 1997; Giordano *et al.* 2004). Raised levels of AGP have also been reported in tumour-bearing cats (Selting *et al.*, 2000) including those with lymphoma (Correa *et al.*, 2001). Determination of the SAA concentration may also be useful in cats, as it was shown to be the most rapidly responding APP in a variety of inflammatory and infectious conditions (Kajikawa *et al.*, 1999). The cat is a species where CRP does not show a major response in contrast to the situation in dogs. Haptoglobin concentrations are raised in the feline acute phase response though further investigation is required to determine the full diagnostic value of this protein.

TABLE 3: Feline diseases where an acute phase response has been described (Ceron *et al.* 2005).

Acute Phase Protein	Disease/condition
AGP	Anaemia of inflammatory diseases Feline coronavirus Feline calicivirus Feline chlamydiosis Feline leukaemia virus Feline infectious peritonitis Feline immunodeficiency virus Lymphoma Surgery Tumours Miscellaneous infections
Haptoglobin	Anaemia of inflammatory diseases Feline infectious peritonitis Splenectomy Surgery Miscellaneous infections
SAA	Diabetes Feline infectious peritonitis Renal failure Urinary tract disease Surgery Miscellaneous infections

Although there is compelling evidence that APPs are valuable biomarkers for disease in veterinary medicine it is important that the use and interpretation of the values generated by tests for these biomarkers of disease is understood especially as the tests are becoming more generally available with canine CRP, feline AGP and haptoglobin in both species being available as a routine service (ReactivLab Ltd, www.reactivlab.com).

It is likely that within a few years the APP tests will become a permanent and frequently requested diagnostic test within small animal medicine.

INTERPRETING ACUTE PHASE PROTEIN PROFILES IN PRACTICE: A CLINICIAN'S PERSPECTIVE

As outlined above, acute phase protein assays are becoming increasingly available, and there is no doubt that they can serve as an extremely useful diagnostic tool. When interpreting acute phase profiles in an individual patient, it is helpful to keep several key points in mind.

Acute phase proteins are very sensitive markers of inflammation/infection

Almost any inflammatory, immune-mediated or infectious disease will produce a measurable and frequently dramatic increase in APP concentrations. Acute phase protein concentrations are more sensitive than leukocyte counts in detecting inflammatory or infectious diseases. There are two implications of this sensitivity:

Acute phase proteins are non-specific

Increased APP concentrations identify the presence of an inflammatory focus, but they do not indicate which body system(s) is affected, or what the specific disease entity is. Acute phase proteins simply act as 'markers' for the presence of inflammatory disease and need to be interpreted in conjunction with a thorough clinical exam and any other diagnostic tests performed.

- **'Major' and 'minor' APPs will behave differently in response to an inflammatory stimulus**

Circulating concentrations of the major APPs will rise earlier, and to a much greater extent than will concentrations of the minor APPs. Following resolution of the disease process that stimulated the rise in APPs, concentrations of the 'major' APPs will return to baseline earlier than the 'minor' APPs. Variations in the relative concentrations of major and minor APPs in an individual patient over time can help provide information regarding the resolution or chronicity of a condition. This is one of the reasons why it is useful to include at least one major and one minor APP in the APP profile.

- **Glucocorticoids increase haptoglobin concentrations in dogs**

Haptoglobin is a minor acute phase protein in dogs, concentrations of which can be increased by either exogenous administration, or

endogenous production of glucocorticoids. In a dog with Cushing's disease, or a dog receiving glucocorticoids, a mild neutrophilia and a mild increase in haptoglobin concentrations would be expected, however an increase in C-reactive protein (a major APP in dogs) would indicate the presence of a concurrent infectious or inflammatory disease.

Clinical utility of acute phase proteins

Bearing in mind the principles of APP interpretation outlined above, it is evident that APP profiles have a number of potential uses in practice:

- Due to their sensitivity, acute phase proteins can be used to detect subclinical disease (e.g. as part of a health screen)
- In an animal with clinical signs that might have either inflammatory or non-inflammatory aetiologies, APPs can help determine the role of inflammation in the disease process.
- In an animal with a definitive diagnosis of a specific inflammatory disease, serial monitoring of APP concentrations can help determine the response or otherwise of the disease to therapy.

Some of these uses are illustrated in the case example below.

CASE EXAMPLE - USING ACUTE PHASE PROTEINS IN PRACTICE

'Ben' is a 5-year-old male Cocker Spaniel. He presented with a history of progressive dyschezia and haematuria. A diagnosis of acute prostatitis and an *E. coli* bacterial cystitis was reached following workup. Antibiotic therapy was commenced, and, as castration was declined by his owners at this time, an injection of delmadinone acetate (Tardak; Pfizer) was administered.

Ben's clinical signs rapidly abated (by week 2 of therapy). The gradual fall in APP concentrations (Fig. 2) mirrored his clinical improvement and was further evidence that his inflammatory disease was

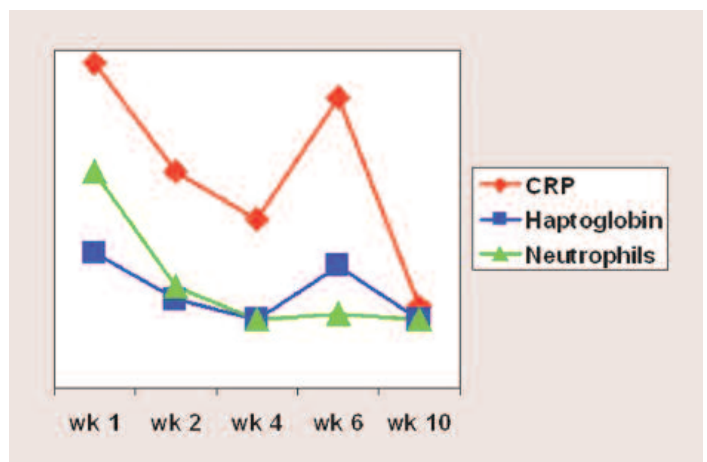


Fig. 2: Graph of Ben's circulating concentrations of CRP, haptoglobin and neutrophil count over time. The shaded area represents the normal range. Values are depicted as multiples of the upper limit of the normal range for each variable.

resolving. By week 4, repeat urine cultures showed a resolution of the bacterial cystitis, so despite the ongoing elevation of CRP concentrations, antibiotic therapy was stopped. By week 6, although Ben remained free of any clinical evidence of disease, and his neutrophil count was within normal limits, APP concentrations had rebounded, suggesting the presence of subclinical inflammation. In light of the lack of evidence of inflammatory disease involving body systems other than the urogenital tract, a repeat urine culture was performed which showed a recurrent bacterial cystitis. Antibiotics were recommenced and at this point, his owners consented to having Ben castrated. Four weeks later, with acute phase protein concentrations within normal limits, antibiotic therapy was stopped. Repeat urine cultures failed to yield any bacterial growth. Follow up APP profiles were persistently within normal limits and Ben is currently free of any clinical signs of disease.

This example shows how APPs can detect subclinical disease. The increased APP concentrations at week 4 probably reflected an ongoing bacterial prostatitis. It may have been prudent to continue antibiotic therapy and proceed with castration at that time. In a patient with a defined inflammatory disease, the subsequent rise in APP concentrations suggested that a relapse of clinical signs was a possibility and prompted the repeat urine culture, which confirmed the suspicion of progressive inflammatory/infectious disease. As our knowledge of the clinical applications of APPs improves, it is possible that they may provide a relatively cheap, non-invasive alternative to other conventional diagnostic tests.

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