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Early Biomarkers of Inflammation in Dogs and Cats: The Acute Phase Proteins

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Abbreviations: AGP, α_1-acid glycoprotein; APP, Acute Phase Proteins; APR, Acute Phase Reaction; CBP, Cortisol Binding Protein; Cp, Ceruloplasmin; CRP, C-Reactive protein; Hp, Haptoglobin; IL-1, interleukin 1; IL-6, interleukin 6; LBP, Lipopolysaccharide Binding Protein; RBP, Retinol Binding Protein; SAA, Serum Amyloid A; TNF-α, Tumor Necrosis Factor α; TTR, Transthyretin

THE ACUTE PHASE REACTION AND THE ACUTE PHASE PROTEINS

During local or systemic inflammation phagocytes within the inflamed tissues produce cytokines such as interleukin 1 (IL-1), interleukin 6 (IL-6) and Tumor Necrosis Factor α (TNF-α). These cytokines are released into systemic circulation and induce a sequela of responses known as the "acute phase reaction" (APR), involving different homeostatic systems. The final aim is to establish rapid and intense stimulation of a protective response. One of the main aspects of the APR is the modulation of protein synthesis by hepatocytes (Ceciliani *et al*., 2002). As a consequence, the concentration of some plasma proteins, which are called *Acute Phase Proteins* (APP), vary more than 25% compared to the basal level. The large majority of APP are *positive APPs*, since their concentration in blood increases during inflammation, but "negative APPs" (APPs whose concentration in blood decreases) also exist. As previously mentioned, the modulation of protein production by the liver depends on the synergic action of the cytokines mentioned above: TNF-α induces peripheral proteolysis, thus increasing the flow of aminoacids to the liver; IL-1 inhibits the synthesis of negative APPs and stimulates the synthesis of positive APPs in collaboration with glycocorticoids, also produced as a consequence of IL-1-mediated activation of the pituitary-adrenal axis; IL-6 is involved in the release of positive APPs from hepatocytes.

Albumin is the main negative APP. Albumin is the most abundant protein in blood and a large amount of aminoacids is required for its production: during APR decreased synthesis of albumin increases the amount of available aminoacids which are required for the synthesis of positive APPs. Other negative APPs are the Retinol Binding Protein (RBP), the Cortisol Binding Protein (CBP) and Transthyretin (TTR), which transports hormones or vitamins. When these transport proteins decrease, most of the circulating

hormones or vitamins are released into circulation and, since only these "free" forms are biologically active, the organism can thus better utilize hormones and vitamins without increasing their production.

Positive APPs include proteins with antibacterial or immunomodulating activity (C-Reactive protein or CRP, complement fractions such as C3 and C4, α_1-acid glycoprotein or AGP, LPS Binding Protein or LBP); transport proteins (Haptoglobin or Hp, which binds Hemoglobin, Ceruloplasmin or Cp, which has immunomodulating functions and binds copper); scavenger proteins which protect tissues from potentially dangerous molecules (Serum Amyloid A or SAA, α-globulins with antiproteasic activity such as α_1-antitripsin). Some of the positive APPs (C3, C4, fibrinogen) only show moderate increases during inflammation, some APPs (Hp, AGP) increase 2–5 times compared to the basal level amd others (SAA) increase 100 to 1000 times. In dogs and cats the "major" acute phase proteins (those that increase more frequently and are thus the marker of choice in diagnosis) are CRP and AGP, respectively.

Most of the APPs migrate within α-globulins and serum protein electrophoresis may indicate the occurrence of an APR. By contrast, colorimetric or immunologic (ELISA, immunoturbidimetry, radial immunodiffusion) methods are required to measure single APPs. These methods can create problems, since species-specific anti-feline or anti-canine antibodies are rarely available and are very expensive, when available.

APPs IN ROUTINE PRACTICE: ADVANTAGES AND LIMITATIONS

APP production is rapid (it takes only few hours to be detectable) and intense but not specific for a given disease. The above mentioned cytokines, in fact, are induced by any inflammatory stimulus (independent of its type and severity) or even by non inflammatory stimuli, such as tumours or pathophysiologic conditions (stress, pregnancy). This lack of specificity represents a significant limitation (that can be by-passed as demonstrated below) but also an advantage from a diagnostic point of view, since the increase of these proteins means that the organism is combating a potentially dangerous event. Basically, the measurement of positive APPs has the opposite role of serology, bacteriology and clinical pathology techniques which demonstrate that a specific pathogen has encountered the organism and/or damaged some system or apparatus (e.g. causing anemia, renal failure, etc). On the contrary, high APP levels do not help us understand which pathogen is (or was) present, but demonstrate that the organism is still fighting against a pathogen. On this basis, in human medicine the measurement of APP levels has gained the role of a prognostic and clinical decision making marker. APPs are also used when the diagnosis of a specific disease has already been obtained by routine clinical or clinico-pathological approaches, to establish whether the observed changes are a sequela of host-pathogen interaction (APP levels are likely to be low) or if the organism is still responding to a potential pathogen (APP levels would be high and it might be necessary to change or modulate the therapeutic approach). Also, in human medicine APPs are used during follow-up to monitor the response to therapies: when treatments are effective, APPs return to baseline values more rapidly than other indicators of disease regression. Finally, APPs may also induce diseases by themselves: for example, the

SAA produced during non specific inflammation extravasates from blood to tissues to remove oxidized lipids and then it is proteolized by tissue enzymes. When inflammation persists, SAA saturates tissue proteolytic enzymes: partially proteolysed SAA assumes a β-sheet conformation and accumulates in tissues as amyloid (reactive systemic amyloidosis). Similarly, SAA fibrils accumulate in tissues during hereditary amyloidosis of Sharpei dogs, Oriental, Abyssinian or Somali cats, which have amyloidogenic SAA sequences which cannot be completely proteolyzed. Both reactive systemic and hereditary amyloidosis can be monitored by measuring serum SAA.

The main limitations of APP measurement are the poor diagnostic specificity mentioned above and the relatively high costs per test. This latter aspect will be probably by-passed in the future, since several papers (Kjlelgaard-Hansen *et al*., 2003 as an example) have validated automated methods which would decrease the cost per test and allow simultaneous processing of numerous samples. As regards the poor diagnostic specificity, future studies would define the optimal cut-off to discriminate animals with inflammations from those with diseases other than inflammation.

DIAGNOSTIC UTILITY OF APPs IN CANINE MEDICINE

As regards APP measurement in dogs, several studies have revealed both the role of APPs as markers of inflammation and the level of single APP in specific disorders. Generally speaking, blood levels of major APPs in dogs (CRP, SAA, and, to a lesser extent, Hp and Cp) are much more elevated in dogs with inflammation, even if these are non specific or induced by surgery, than in dogs with tumours, degenerative or immune-mediated disorders (Conner *et al*., 1988; Solter *et al*., 1991; Ogilvie *et al*., 1993; Hayashi *et al*., 2001; Martinez-Subiela *et al*., 2003; Fransson *et al*., 2004; Tecles *et al*., 2005). Moreover, APPs have been used for prognostic or monitoring purposes. For example, dogs with canine babesiosis (diagnosed by conventional hematologic approaches) have higher APP levels when complications are present (Lobetti *et al*., 2000). Similarly, CRP and AGP levels increase during pre-symptomatic phases of erlichiosis and leishmaniasis (Rikihisa *et al*., 1994; Martinez-Subiela *et al*., 2002). Moreover, canine APP levels decrease early after appropriate treatment, independent of the type of disease, but remain elevated in "poor responders" (Lobetti *et al*., 2000; Tecles *et al*., 2005). This confirms the role of APPs in treatment monitoring in dogs. Finally, APP levels can be influenced by vaccination, by steroid or barbiturate therapy or during pregnancy (Eckersall *et al*., 1993; Hojo *et al*., 2002; Martinez-Subiela *et al*., 2004). In these situations it is thus possible to have "false positive" increases of APPs.

DIAGNOSTIC UTILITY OF APPs IN FELINE MEDICINE

Data regarding APP levels in cats are scarce. It has been demonstrated that AGP, Hp and SAA increase 24 hours after inflammation or surgery, independent of the presence of possible post-surgical complications (Kajikawa *et al*., 1999). SAA levels increase also during renal failure, neoplasms and liver disorders (Sasaki *et al*., 2003). Similarly,

AGP increases in cats with lymphoma (Selting *et al*., 2000). Most of the studies on feline AGP, regard feline infectious peritonitis (FIP), a lethal disease that is difficult to diagnose by conventional approaches: AGP increases in FIP are so evident to be diagnostic for this disease (Duthie *et al*., 1997). Nevertheless, it has been demonstrated that non-symptomatic shedders of feline coronavirus (the virus responsible for FIP) can also exhibit cyclic fluctuation of serum AGP levels, most likely due to continuous re-infection (Giordano *et al*., 2004). Based on this finding, AGP levels should be interpreted with caution in cats living in endemic catteries.

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