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ACUTE PHASE PROTEIN - A USEFUL MARKER OF INFLAMMATION

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The acute phase response is a nonspecific inflammatory reaction of the host that occurs shortly after any tissue injury. The response includes changes in the concentration of plasma proteins called acute phase proteins (APPs), some of which decrease in concentration (negative APPs), such as albumin or transferrin, and others of which increase in concentration (positive APPs), such as C-reactive protein, serum amyloid A, haptoglobin, alpha-1-acid glycoprotein, and ceruloplasmin. Most positive APPs are glycoproteins synthesized mainly by hepatocytes upon stimulation by proinflammatory cytokines and released into the bloodstream. The acute phase response and clinical application of monitoring APPs in dogs and cats are described, including biochemical characteristics, assays developed for each individual APP, and preanalytic and analytic factors influencing APP results that should be taken into account for proper and adequate clinical interpretation. In addition, the diagnostic use of APPs and their possible application in monitoring treatment, which can be considered one of the most interesting and promising practical applications of these proteins. Finally, challenges and future developments of APPs in dogs and cats will be considered, because it is expected that new and cheaper automated assays for determination of the main APPs in small animals will contribute to a wider use of these proteins as biomarkers of infection and inflammatory lesions.

Key words: Acute phase protein, Infalmmation

The acute phase response refers to a nonspecific and complex reaction of an animal that occurs shortly after any tissue injury. The origin of the response can be attributable to infectious, immunologic, neoplastic, traumatic, or other causes, and the purpose of the response is to restore homeostasis and to remove the cause of its disturbance (Ebersole and Cappelli, 2000). The acute phase response is considered a part of the innate host defense system, which is responsible for the survival of the host during the critical early stages of attack, and in evolutionary terms, it predates the acquired immune response (Eckersall, 2000). The acute phase response is characterized by a number of different systemic effects, including fever, leukocytosis, increased blood cortisol and decreased thyroxine concentrations, metabolic changes (ie, lipolysis, gluconeogenesis, muscle catabolism), and decreased serum iron and zinc concentrations. The response also includes changes in the concentrations of plasma proteins, called acute phase proteins (APPs) (Kushne Kushner and Mackiewicz, 1993). some of which decrease in concentration (negative APPs; eg, albumin or transferrin) and others of which increase in concentration (positive APPs; eg, C-reactive protein [CRP], serum amyloid A [SAA], haptoglobin [Hp], alpha-1-acid glycoprotein [AGP], ceruloplasmin (Cp), and fibrinogen). Most positive APPs are glycoproteins synthesized mainly by hepatocytes upon stimulation by proinflammatory cytokines and released into the bloodstream.

BIOCHEMICAL PROPERTIES AND METHODS OF MEASUREMENT

Biochemical properties and different methods of analysis for each individual APP will be described as, in many cases, biochemical characteristics influence methods of measurement. For laboratories that are currently unable to set up specific methods for APP measurement, it can be useful to note that most APPs migrate to the α- and β-globulin areas, so routine electrophoresis on agarose or cellulose acetate gels can be used to identify overall increases in APP concentrations in inflammation. However, this method is much less sensitive than the individual APP assays. C-reactive protein: When bound to bacteria, promotes the binding of complement, which facilitates bacterial uptake by phagocytes. It has been considered as a primitive form of antibody specifically interacting with cell membrane components of microorganisms. It causes induction of cytokines. Inhibition of chemotaxis and modulation of neutrophil function also occur due to this protein. Canine CRP has a molecular weight of 100 kD, which consists of 5 subunits of 20 kD each. This protein was the first APP to be described. Originally named for its ability to bind the C-polysaccharide of Pneumococcus pneumoniae, CRP has been defined in humans as an exquisitely sensitive systemic marker of inflammation and tissue damage (Pepys and Hirschfield, 2003). Examined by electron microscopy, canine CRP resembles human CRP; the main difference between the proteins is that 2 of the 5 subunits of canine CRP are glycosylated, (Caspi et al., 2003) which could explain in part the difficulties of using antibodies raised against human CRP for canine measurements (Parra et al., 2002).

Measurement of serum CRP is generally by immunoassays using specific canine CRP antibodies, and several formats have been developed and described for this purpose, such as an immunoturbidimetric assay adapted for automated biochemical analyzers, (Eckersall et al ., 1991) ELISA or slide/capillary reverse passive latex agglutination tests. New methods based on time-resolved fluorometry (TRFIA) have been recently developed for CRP assays in canine whole blood, (Parra et al., 2005) saliva,(Parra et al., 2005) and effusions (Parra et al., 2005). Recently, a rapid assay giving qualitative results by sample dilution followed by immunochromatography on a prepared test strip has been produced for canine CRP which could differentiate between samples with CRP concentrations of ,5mg/L and 0.5mg/L. False positive results are a major limitation of this test. To our knowledge, information about the biochemical properties and methods of measurements of CRP in cats is scarce because CRP does not seem to be involved in the acute phase response in this species (Kajikawa et al., 1999).

Serum amyloid-A: It causes chemotactic recruitment of inflammatory cells to sites of inflammation. Produce down regulation of the inflammatory process (inhibition of myeloperoxidase release and inhibition of lymphocyte proliferation). Its involvement also observed in lipid metabolism and transport.

SAA is a small serum protein with a molecular weight of 15 kD. It is thought to be the precursor of amyloid protein A, the major protein of a-amyloid, so it is potentially involved in the pathogenesis of amyloidosis and other chronic inflammatory diseases such as rheumatoid arthritis (Uhlar and Whitehead, 1999). Its use in animals has, until recently, been limited due to difficulties in purification and quantification, probably because it is a hydrophobic apolipoprotein that is complexed within serum high-density lipoproteins (Horadagoda et al., 1999).

Amyloid protein A from dogs and humans shows considerable homology, (Hold and Gruys,1984) although the primary structure of canine SAA has an additional peptide of 8 amino acids (Sellar et al., 1991). It seems the sequence and inductive capacity of SAAs are highly conserved across evolutionarily-distinct vertebrate species (Syversen et al., 1994).

Specific sandwich ELISAs using anti-canine (Yamamoto et al., 1994) and antifeline (Sasaki et al., 2003) SAA antibodies for canine and feline SAA measurements, respectively, have been developed, although technical difficulties in the preparation of antiserum to canine SAA have been reported (Yamamoto et al., 1994). Monoclonal antiserum against human SAA has been successfully used in a sandwich ELISA to measure canine SAA, (Yule et al., 1997) and polyclonal antiserum against canine SAA has been used to detect feline SAA (Kajikawa et al., 1996) In addition, a commercially available ELISA for SAA determination in veterinary species using monoclonal antiserum against human SAA has been proven to be useful for canine (Martý´nez et al., 2005) and feline (Giordano et al., 2004) SAA

quantification; however, technical improvements are needed to reduce between-run imprecision.

Haptoglobin: Its Involvement found in host defense responses to infection and inflammation. Acts as a natural antagonist for receptor-ligand activation of the immune system. Binding of free hemoglobin (a toxic and proinflammatory product resulting from hemolysis). Bactericidal effect in infected wounds by binding hemoglobin and limiting the availability of hemoglobin iron for bacterial growth. It produce inhibition of granulocyte chemotaxis and phagocytosis.

Dogs have only 1 subtype of Hp compared with humans, who have 3 subtypes (Hp 1-1, Hp 2-1, and Hp 2-2). However, canine Hp closely resembles human Hp 1-1 with respect to amino acid content, molecular weight (81 kD), electrophoretic pattern on starch gel, and the existence of a and b subunits in a tetra-chain arrangement (b-a-a-b). (Shim et al., 1971). Compared with human Hp 1-1, canine Hp has 2 structural differences: (Mominoki et al., 1995) the 2 ab chains are joined by a noncovalent interaction rather than by a disulfide bridge (this noncovalent linkage also exists in feline Hp), and the a-chain has an oligosaccharide-binding sequence and is glycosylated, whereas the human a-chain is nonglycosylated. These structural particularities may be responsible for the divergence of findings in the recognition of canine and feline Hp by antibodies directed against human Hp. For example, it has been reported that a monoclonal antibody against human Hp did not recognize canine and feline Hp, but this antibody was directed to the disulfide bond linking the Hp chains that is not present in dogs and cats (Katnik et al., 1998). The glycosylation pattern of Hp can vary in dogs with various inflammatory, autoimmune, and neoplastic diseases (Andersson et al., 1998). Assays for serum Hp concentration can be divided into 2 main groups: a) spectrophotometric assays and b) immunoassays. Different spectrophotometric manual assays have been based on the ability of Hp to bind hemoglobin (Hb), forming Hp–Hb complexes that either alter the absorbance characteristic of Hb in proportion to the concentration of Hp in a serum sample or preserve peroxidase activity at an acidic pH, which then can be detected and quantified. In addition, an automated spectrophotometric multispecies assay based on the peroxidase activity of Hp–Hb complexes, in which interference by serum albumin is eliminated, has been described and validated at different laboratories for use with canine serum, giving satisfactory results (Eckersall et al., 1999).

Alpha-1-acid glycoprotein: It is an Antiinflammatory and immunomodulatory agent with antineutrophil and anticomplement activity. It increases the secretion of interleukin-1 receptor antagonist by macrophages. Drug binding to numerous basic and neutral lipophilic drugs and also acidic drugs, such as phenobarbital.The main biochemical characteristic of AGP is that it is a highly glycosylated protein and is the main protein component in seromucoid, the fraction of plasma that is most resistant to acid precipitation. (Eckersall, 2000). Canine AGP has been purified and biochemically characterized and, similar to AGP in humans. Although AGP can be estimated by precipitation of the majority of serum proteins by perchloric acid and quantification of the remaining soluble proteins,(Eckersall et al., 1999) this protein usually is measured by single radial immunodiffusion on agarose gel impregnated with anti-species AGP rabbit serum, using different commercial kits. These tests are species-specific and are currently available for dogs and cats; however, they have the disadvantage of requiring 24 or 48 hours for diffusion to be complete (Eckersall et al., 1999). Immunoturbidimetric assays for the measurement of canine and feline AGP have been developed,(Kuribayashi et al., 2003) offering the advantages of being rapid and adaptable to biochemical analyzers. Ceruloplasmin: Transport of copper needed for wound healing, collagen formation, and maturation. Protection of cells and tissues against oxidant compounds generated by phagocytes in the course of clearing microorganisms or tissue debris. Reduction in the number of neutrophils attaching to endothelium.

Ceruloplasmin is an α2-glycoprotein and one of the positive APPs in dogs (Martinez et al., 2001). Although there are no reports about the structure of canine or feline Cp, studies of human Cp have shown that it is a blue protein with a molecular weight of 151 kD containing about 0.34% copper, which corresponds to 8 atoms of copper per molecule. Additionally, it is a glycoprotein containing hexosamine, hexose, and neuraminic acid(Rice, 1963).

NEGATIVE ACUTE PHASE PROTEINS:

Albumin is the most abundant protein in blood, constituting 35–50% of protein in the plasma of healthy dogs and cats; it is the major band observed in serum protein electrophoretograms. Albumin is responsible for about 75% of the osmotic pressure of plasma and is a major source of amino acids that can be utilized by the animal's body when necessary. Albumin usually is measured in routine practice by spectrophotometric

methods, such as the bromcresol green assay; however, overestimation of albumin can occur in heparinized plasma samples assayed by this method (Stokol et al., 2001).

Transferrin is a plasma glycoprotein that is responsible for the transport of iron in the circulation; it has a single polypeptide chain of about 700 amino acids. Transferrin binds iron as a ferric ion in 2 binding sites at a neutral pH, but the ion is dissociated when the pH falls below 5.5 (Smith, 1997). Although total transferrin can be measured by immunoassay, it is commonly assessed by measuring the total iron-binding capacity (TIBC) of serum, though in dogs and cats this test has been used largely for the assessment of iron metabolism and homeostasis (Smith, 1997).In humans, there has been debate on the balance between nutritional and disease effects on the production of negative APPs (Ingenbleek and Carpentier, 1985). A recent hypothesis is that the acute phase response has a stronger effect than the nutritional plane on concentrations of transferrin, albumin, and other negative APPs (Fuhrman et al., 2004).Thus, a decreased concentration of transferrin may be more indicative of an acute phase response than of poor diet.

CLINICAL VALUE OF ACUTE PHASE PROTEIN DETERMINATION:

Classical APPs, such as albumin and fibrinogen, although currently easier and cheaper to measure, seem to have a lower clinical value in diagnosing and monitoring inflammation. For example, a decreased albumin/globulin (A/G) ratio provides an estimate of the acute phase reaction in infection or inflammation in dogs and cats, (Kaneko, 1997) because of decreased albumin concentration (as a negative APP) and increased globulins concentration. However, the sensitivity and specificity of the ratio for detecting clinical or subclinical disease are not as high as those of positive APPs such as CRP (Martý´nez et al., 2002). Similarly, in cats with FIP, the A/G ratio has been a valuable diagnostic test for this viral infection for some time, but the use of a positive APP, AGP, has been shown to have superior diagnostic efficiency (Duthie et al., 1997). Although the pathophysiologic reaction of fibrinogen to infection and inflammation has been known for many years and occurs in dogs and cats, as in other mammalian species, (Kaneko, 1997) fibrinogen has not been routinely measured in veterinary diagnostic laboratories as an acute phase protein. Indeed, most of the interest in measurement of fibrinogen in canine medicine has been for the diagnosis of disseminated intravascular coagulation and hyperfibrinolysis, in which the concentration of fibrinogen falls (Mischke et al., 1998). In a study of APPs in 161 dogs with inflammation, the predictive value of a positive or negative test for Hp and Cp was comparable to or better than that of fibrinogen (Solter et al., 1991). Thus, the use of fibrinogen as an APP in dogs and cats appears to largely have been superseded by the growing availability of specific assays for APPs that show greater and more rapid responses on stimulation than the 2- to 4-fold increase observed in the concentration of fibrinogen.

MAGNITUDE AND TIME COURSE OF THE ACUTE PHASE RESPONSE:

Acute phase proteins in dogs and cats can be classified by the magnitude of their response to stimuli as major (10- to 100-fold increase), moderate (2- to 10-fold increase), or negative (decrease) reactants. For major APPs, especially in cats, upper limits of increase are lower than those described for humans, who experience increases as high as 1000-fold.

In dogs, differences in the magnitude and also the time course of response

have been detected between major and moderate APPs . Major APPs (CRP and SAA) usually have an early and high rise in concentration and a very rapid decline. The CRP increase in dogs is indeed more rapid than in humans because, in the latter species, levels do not increase until after 6 hours. Although no data were given about the half-life of CRP and SAA in dogs and cats, it appears that the half-life of canine CRP is short (Conner et al., 1988). The rapid production and clearance of CRP make it a very useful test to indicate the clinical situation of an animal at the time of sample collection (Caspi et al., 1984). In humans, SAA is catabolized in the liver and has a half-life of 1 day, although in acute or chronic inflammation, the capacity of the liver to degrade SAA decreases (Gollaher and Bausserman, 1990).

On the other hand, moderate APPs (Hp, AGP, and Cp) need more time to increase and return to normal values, with a more gradual decline. Increases in Cp concentration in dogs are higher and evidently earlier than in humans, peaking at about double normal values on the fourth day following surgery (Conner et al., 1988). Interestingly, the moderate APPs, which increase less in terms of fold-increase, are in higher concentration than CRP and SAA in the serum of healthy animals, and the total amount of protein produced during the acute phase response is usually higher.

The peak time and magnitude of an APP response can vary depending on the type of stimulus. In dogs that had undergone surgery or were infected with Bordetella bronchiseptica, peak CRP responses were observed within 1 day; however, with infection by intracellular microorganisms, such as Trypanosoma brucei or Ehrlichia canis, the peak appeared at 4–10 days postinfection (Rikihisa et al., 1994). Increases of 95-fold were found in CRP concentrations after surgical trauma compared with 40- to 50-fold increases after turpentine oil injection. Five-fold increases have been found in Hp and Cp concentrations in dogs with leishmaniosis, compared with the 2- to 3-fold increases found with surgical trauma (Conner et al., 1988).

COMPARISON WITH OTHER MARKERS OF INFLAMMATION: Some relationship, although weak, exists between APPs and WBC and neutrophil counts. A significant difference in serum CRP concentrations between clinically normal dogs and dogs with high neutrophil counts was detected, and a significant positive correlation, although with low correlation coefficients, was found between the concentrations of different positive APPs (CRP, Hp, and Cp) and WBC and segmented and band neutrophils counts (Solter et al., 1991). However, there are some advantages of APPs compared with leukocyte counts as markers of inflammation. APPs have higher diagnostic sensitivity. Ceruloplasmin and Hp are up to 6 times more sensitive than leukocyte counts in detecting inflammation, and are increased in cases in which total and differential WBC counts showed no changes (Solter et al., 1991). In addition, APPs concentrations are of value for detecting inflammation in animals in which the bone marrow cannot respond normally to an inflammatory stimulus, such as those with myelosuppresion attributable to treatment with chemotherapeutic agents or those with leukemia (Jain, 1989). Longer analyte stability. Acute phase proteins are more stable than the cellular components of blood, and assays can be performed on frozen serum or plasma samples (Solter et al., 1991). Theoretically, APPs exhibit a faster response than changes in WBC counts, especially in inflammation, where new WBCs must be generated in the bone marrow. However, significant increases in CRP concentration after surgical trauma do not appear earlier than alterations in CBC values (Kjelgaard et al., 2003).

CHALLENGES AND FUTURE DEVELOPMENTS:

Practical uses and advantages of APP assays have been described and demonstrated in a large number of scientific reports published in the last few years. Clinical application of APPs has not been extensive in routine small animal practice, however, due to practical limitations associated with analysis and interpretation. There are insufficient studies on the sensitivity of APPs for the diagnosis of many diseases. In addition, few commercial kits for APP analysis are available for small animal species, and those available are relatively expensive. These assays need to be validated for each use in alternative species; however, the validation procedure should be repeated for each new batch of antiserum (Eckersall et al., 1999). An international standardization project for APPs in farm animals has been developed,(Skinner, 2001) and similar efforts for companion animals are needed.

Despite these challenges, a number of new developments regarding the use of APPs in small animals show promise for the future. Because APPs vary in their response to inflammation and tissue damage, measurement of several APPs in combination or a serum APP profile may be more meaningful than making observations on a single protein (Eckersall et al., 1995). Use of APP profiles involving at least 1 major (CRP or SAA), 1 moderate (Hp, AGP, or Cp), and 1 negative APP are likely to become more widely used instead of the determination of individual APPs. For example, the combination of a fast APP, such as CRP, and a slow one, such as Hp, has been recommended to differentiate between pathologic states (Eckersall, 2003) and to provide more information about the temporal evolution of the disease. Additional comparative studies involving the simultaneous determination of various APPs would be of great help in deciding which major, moderate, and negative APPs are the most applicable for clinical use in specific diseases. Development and use of novel drugs that specifically block the in vivo proinflammatory effects of APPs, such as CRP or SAA, have shown promise in initial investigations (Pepys et al., 2002).Such drugs could be used to avoid complications of the acute phase response, such as amyloidosis.

It is expected that, in the near future, with the development of new and cheaper tests and the harmonization of assays, APPs could be more widely used in routine small animal practice. APPs have proven to be very useful in the early detection of subclinical diseases or alterations of the health status of an animal, with predictive information regarding the development of disease in the future. Changes in serum APP concentrations indicate the need for a more detailed clinical evaluation of a patient. In addition, APPs can be a powerful tool in patient management and the monitoring of treatment. As has been pointed out recently, in the future, any health check regime that omits evaluation of the APP response could be considered less than optimal (Eckersall, 2004).

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