# Alterations in serum protein electrophoresis profiles during the acute phase response in dogs with acute pancreatitis

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## Abstract

The quantification of serum proteins is a useful tool for diagnosing and monitoring various diseases that involve changes in the concentrations of these proteins. As canine acute pancreatitis (AP) accompanies the systemic inflammatory response syndrome, serum proteins such as C-reactive protein (CRP) have been used as inflammatory markers for dogs with AP. The goal of this study was to investigate the overall profiles of serum proteins by serum protein electrophoresis (SPE) and to determine the concentration of acute phase proteins (APPs) in dogs with AP in order to better understand serum protein profiles as diagnostic markers in these dogs. Decreased levels of albumin and increased levels of alpha-2 globulin were observed in dogs with AP by SPE. Among APPs, elevated concentrations of CRP, serum amyloid A (SAA), and haptoglobin were detected. The concentration of SAA was positively correlated with that of CRP, which suggests that SAA could be a sensitive marker of inflammation in dogs with AP, similar to CRP.

## *Résumé*

*La quantification des protéines sériques est un outil utile pour diagnostiquer et suivre différentes pathologies qui impliquent des changements dans les concentrations de ces protéines. Comme la pancréatite aiguë (AP) accompagne le syndrome de réponse inflammatoire systémique, les protéines sériques telles que la protéine C-réactive (CRP) ont été utilisées comme marqueurs d'inflammation chez les chiens avec AP. L'objectif de la présente étude était d'examiner les profils globaux des protéines sériques par électrophorèse des protéines sériques (SPE) et de déterminer les concentrations des protéines de phase aiguë (APPs) chez les chiens avec AP afin de mieux comprendre les profils de protéines sériques comme marqueurs diagnostiques chez ces chiens. Des niveaux diminués d'albumine et des niveaux augmentés de globuline alpha-2 furent observés par SPE chez des chiens avec AP. Parmi les APPs, des concentrations élevées de CRP, d'amyloïde sérique A (SAA), et d'haptoglobine furent détectées. La concentration de SAA était corrélée positivement avec celle de CRP, ce qui suggère que SAA pourrait être un marqueur sensible d'inflammation chez les chiens avec AP, de manière similaire à la CRP.*

*(Traduit par Docteur Serge Messier)*

Serum proteins serve many different functions, including the transport of lipids, hormones, vitamins, and minerals for the regulation of cellular activity (1). They are also involved in regulating the immune response, inflammation, protecting against infection, and repairing damaged tissue. The profiles of serum proteins can be altered by several factors, including the acute phase response, which is an early defense system activated by trauma, infection, and inflammation (2). The quantification of serum proteins is, therefore, a useful tool for the diagnosis, prognosis, and monitoring of diseases that involve changes in the concentrations of serum proteins. Serum protein electrophoresis (SPE) separates proteins by size and electrical charge that are broadly fractionized as albumin and alpha, beta, and gamma (α, ß, and γ) globulins. These fractions are known to contain various proteins that function as part of the acute phase and acquired immune responses (1).

Acute phase proteins (APPs) are blood proteins that are produced by the acute phase response to infection, inflammation, or trauma. They play a role in both a pro- and anti-inflammatory effect on balance between the 2 functions (3). Blood concentrations of APPs have been used as diagnostic and prognostic markers in humans and animals (4). Acute phase proteins (APPs) are classified into categories according to the severity of the acute phase response, corresponding to a major, moderate, and minor response.

Major APPs in dogs include C-reactive protein (CRP) and serum amyloid A (SAA) (3,5). C-reactive protein (CRP) plays a role in the induction of cytokines, inhibition of chemotaxis, and modulation of neutrophil function (3), and is frequently used as a marker for systemic inflammation. Its serum concentration can increase rapidly up to 100-fold as part of the response to a number of infectious and inflammatory diseases in dogs (3,6). Serum amyloid (SAA) also induces chemotactic recruitment of inflammatory cells to sites of inflammation and increases during the acute phase response in dogs, as previously reported for infectious diseases, such as parvovirus infection and leishmaniosis and inflammatory diseases (3–5).

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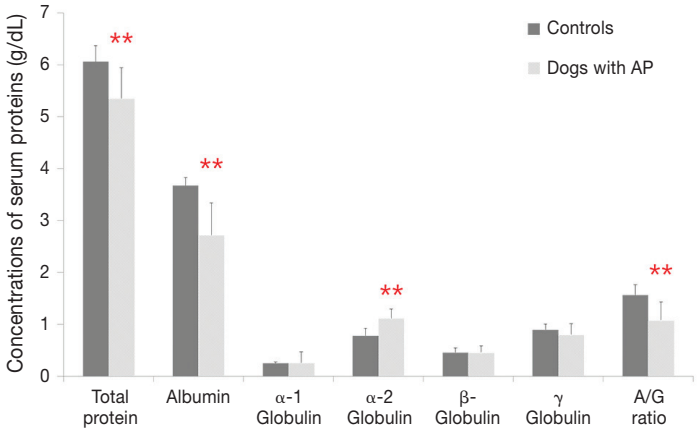


**Figure 1. Concentrations of serum proteins separated by serum protein electrophoresis (SPE). Total protein levels were significantly decreased in dogs with acute pancreatitis (AP). In addition, significantly decreased levels of albumin and significantly increased levels of α2 globulin were observed in dogs with AP. The albumin/globulin (A/G) ratio was also significantly decreased in dogs with AP (**$P < 0.01$).**

Moderate APPs in dogs include haptoglobin and acid glycoprotein. Haptoglobin acts as a natural antagonist for receptor-ligand activation of the immune system and also inhibits granulocyte chemotaxis and phagocytosis (3). Elevations in haptoglobin levels have been reported in dogs with Cushing disease, leishmaniosis, surgical trauma, and inflammatory diseases such as pyometra and pancreatitis (4,5).

Acute pancreatitis (AP) is a relatively common disorder in dogs, occurring predominantly in middle-aged, obese, female dogs. As the clinical condition of canine AP varies from mild to severe, it is important to determine whether the condition of a patient is severe or mild for an accurate diagnosis and appropriate treatment (7). As acute pancreatitis (AP) is accompanied by sudden inflammation of the pancreas, with the adjunctive tissue involved to varying extents (7), there have been attempts to determine whether inflammatory markers such as CRP can be used as diagnostic and prognostic markers for canine pancreatitis. Few studies, however, have investigated the overall profiles of serum proteins in dogs with pancreatitis (1).

The goal of the present study was to investigate the concentrations of overall serum proteins in dogs with AP in order to better understand serum protein profiles as diagnostic markers in these dogs. We identified serum protein fractions differentially represented in SPEs of dogs with AP and further analyzed the concentrations of single APPs, including CRP, SAA, and haptoglobin.

The study was conducted on the serum samples of 13 dogs (6 females and 7 males, 2 to 12 y old) diagnosed with AP by physical examinations (with clinical signs of anorexia, diarrhea, and vomiting), lab analyses, including the SNAP canine pancreas-specific lipase (cPL) kit (IDEXX, Westbrook, Maine, USA), and diagnostic imaging. Four Maltese, 3 Yorkshire terriers, 3 Shih Tzus, 1 Pomeranian, and 2 mongrels were enrolled in the study. Increases of canine pancreas-specific lipase were observed in all enrolled dogs. In addition, 6 beagles (3 females and 3 males, 4 to 8 y old) were enrolled as control dogs. At initial presentation to the hospital, 3-mL blood samples were collected by direct jugular venipuncture, placed in a tube, and centrifuged at 1300 × $g$ for 10 min. Serum samples were then frozen immediately at −80°C until assayed.

Total proteins were evaluated by chemistry analyzer (FUJI DRI-CHEM 7000i; Fujifilm, Tokyo, Japan) and SPEs were conducted by agarose gel electrophoresis (Hydrasys2; SEBIA, Lisses, France) with a protein electrophoresis reagent kit [Hydragel protein(e) 15/30; SEBIA] following the manufacturer's instructions; the fractions were subsequently identified by electrophoretograms. The results of the serum protein electrophoresis gels were reviewed and interpreted by a laboratory expert.

As the fractions in SPE contain various proteins including APPs, the concentrations of single APPs, such as CRP, SAA, and haptoglobin, were also measured by commercial colorimetric kits that are validated for dogs (8). Briefly, CRP and SAA were measured using an immunoassay kit (Tridelta Development, Kildare, Ireland) according to the manufacturer's instructions. In addition, haptoglobin was measured by kits for the peroxidase activity of the haptoglobin-hemoglobin complex (Tridelta Development) according to the manufacturer's instructions. The absorbance of samples was measured on a microtiter plate reader (BioTek, Winooski, Vermont, USA) at 450 nm using 630 nm as reference. The values of dogs with AP were compared to those of control dogs using Student's $t$-test, with a $P$-value of $< 0.05$ considered statistically significant. In order to compare each marker, correlations between CRP, SAA, and haptoglobin were also evaluated by Pearson's correlation. Correlation coefficients $(r)$ of $< -0.2$ and $> 0.2$ were considered as displaying weak negative and positive correlations, respectively, and $r$ of $< -0.4$ and $> 0.4$ were considered to display significant negative and positive correlations, respectively.

By SPE, 5 fractions of albumin and α1, α2, ß, and γ globulins were identified in both control dogs and dogs with AP. The percentage of the fractions in SPE was then multiplied by total protein concentration to quantify values for each fraction (Figure 1). Total protein levels were significantly decreased in dogs with AP. In addition, the levels of albumin were significantly decreased and the levels

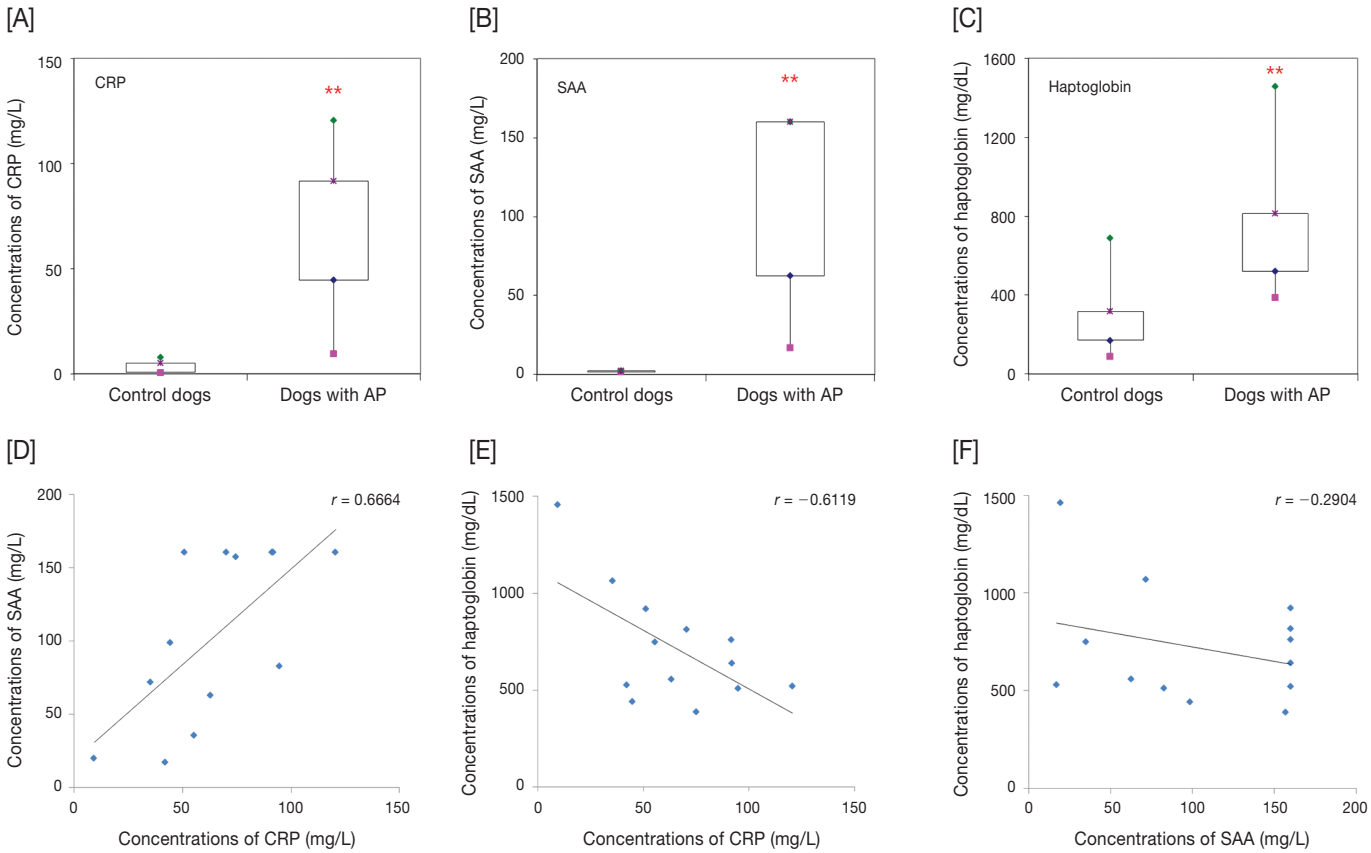


**Figure 2. Concentrations of acute phase proteins and correlations for each protein. Significantly increased concentrations of C-reactive protein (CRP) (A) and serum amyloid A (SAA) (B) and moderately increased haptoglobin (C) were observed in dogs with acute pancreatitis (AP) (**$P < 0.01$). In addition, the concentrations of CRP were positively correlated with those of SAA (D). However, a significant negative correlation with CRP (E) and a weak negative correlation with SAA (F) were observed for the concentrations of haptoglobin in dogs with AP.**

of α2 globulin were significantly increased in dogs with AP. The albumin/globulin ratio (A/G ratio) was also significantly decreased in dogs with AP (Figure 1).

In addition, in order to determine which acute phase proteins were elevated in dogs with AP, the amounts of single APPs were investigated by colorimetric kit. The quantities of CRP, haptoglobin, and SAA in the serum of healthy dogs and dogs with AP are shown in Figure 2. Significant differences in the concentrations of CRP, SAA, and haptoglobin were observed in dogs with AP. C-reactive protein (CRP), which is known as a major APP in dogs, was elevated an average of 20-fold compared to control dogs (Figure 2A). Another major APP, SAA, was elevated in all enrolled dogs with AP, displaying an average elevation of 50-fold compared to controls (Figure 2B). The moderate APP, haptoglobin, was elevated 2-fold in dogs with AP (Figure 2C). Furthermore, for the comparison of each marker, the correlations between the concentrations of CRP, SAA, and haptoglobin were also investigated. A positive correlation was observed between CRP and SAA (Figure 2D, $P = 0.013$), while haptoglobin was negatively correlated with CRP (Figure 2E, $P = 0.027$). A weak negative correlation was also observed between haptoglobin and SAA (Figure 2F, $P = 0.335$).

In this study, the concentration of total protein was significantly decreased in dogs with AP. Decreased albumin synthesis (negative acute phase protein) might contribute to decreased total protein. In addition, increasing protein loss due to gastrointestinal tract or proteinuria because of type-III hypersensitivity glomerulonephritis in AP might be associated with low total protein. The concentration of serum albumin is usually lower in dogs with AP (7), as was also observed in the present study. Decreased albumin levels in dogs with AP are associated with numerous factors, including the preferential synthesis of APP in the liver during the acute phase response (3). When an acute phase response occurs, the liver preferentially synthesizes positive APPs, whereas the production of other proteins, including albumin, is reduced.

By SPE analysis, an elevation of α2 globulin levels was also observed in dogs with AP. As α2 globulin includes haptoglobin, ceruloplasmin, and α2 macroglobulin (5), elevation of haptoglobin concentration might be one of the factors that contributed to increased α2 globulin concentration in dogs with AP. Meanwhile, there was no significant difference in ß globulin, which includes SAA and CRP. It has been reported that increased globulin fractions are observed in SPE when concentrations of APPs such as haptoglobin are increased in the serum (5). Acute phase proteins (APPs) with lower concentrations, such as SAA, may not induce increases in globulin fractions in SPE, even though they are significantly increased in the serum (5). Therefore, SPE analysis in dogs with AP represented characteristic

features in acute phase response, including decreased levels of albumin and increased levels of α2 globulin, although it may be less sensitive in detecting elevations of specific APPs.

In the present study, serum CRP levels were increased up to 20-fold in dogs with pancreatitis. Increased CRP levels in dogs with pancreatitis have been well-described and CRP has been used as a sensitive marker of systemic inflammation in dogs (7,9). However, there are relatively few studies on SAA levels in dogs with pancreatitis (10). It has been reported in humans that SAAs are potentially comparable to CRPs as biomarkers for systemic inflammation (11). In dogs, SAAs were found to be limited as a biomarker for acute phase response, as they were measured with time-consuming methods such as enzyme-linked immunoassays (ELISA) (10), while CRPs could be measured by various point-of-care tests.

A recent study developed a routine measurement of canine SAA using latex agglutination turbidimetric immunoassay (LAT) (10,12) and revealed that significantly higher concentrations of SAA were detected in dogs with systemic inflammation, displaying positive correlation with the concentrations of CRP (12). Both markers showed comparable diagnostic capacity and excellent agreement in clinical classification, indicating that both SAA and CRP were useful diagnostic markers of systemic inflammation in dogs (12). In accordance with that, the present study revealed that increased SAA levels in all enrolled dogs with AP displayed an average elevation of 50-fold, which was positively correlated with CRP concentrations. Pearson's correlation coefficient between canine SAA and CRP was also comparable to those of a previous study (12). These findings therefore suggest that SAA, in addition to CRP, could be a sensitive marker for the detection of inflammation in dogs with AP. It would be expected that SAA could be an alternative to CRP for evaluating the acute phase response of dogs with pancreatitis in future routine clinical practice.

Haptoglobin, a moderate APP, also showed increased levels in dogs with AP compared to controls. The elevation of haptoglobin levels in canine pancreatitis has been reported previously (13,14). Interestingly, the concentrations of haptoglobin were negatively correlated with the concentrations of CRP and SAA in this study. Previous studies that experimentally induced pancreatitis in dogs reported that haptoglobin levels increased slowly compared to CRP levels (9,14). Plasma CRP levels were significantly increased compared to basal values after 3 h of ligation of the pancreatic ducts and peak values were observed after 24 h. The CRP concentrations slowly declined thereafter, but remained high in the plasma until the 96-hour time points (9). Significantly increased levels of haptoglobin were detected, however, after 48 h and peak levels were observed 96 h after ligation of the pancreatic ducts (14). Therefore, if the blood samples in this study were collected after CRP and SAA levels had peaked, the latter declined slowly thereafter. Concurrently, the concentration of haptoglobin began to increase, which may manifest as a negative correlation between concentrations of haptoglobin and those of CRP and SAA. Therefore, CRP and SAA are earlier markers for inflammation in dogs with AP, while haptoglobin increases only slowly.

Pancreatitis needs to be diagnosed using a combination of interpretation of clinical signs in the context of laboratory findings and imaging. While simply measuring serum protein as an indicator of pain is not enough, it may be helpful for monitoring disease progression, response to treatment, and prognosis (7). One of the potential limitations of our study was that concentration of APPs was only measured at its initial presentation. The changes in APP concentrations after dogs have been treated needs to be further investigated to better understand the use of APPs as a prognostic marker in dogs with AP.

Overall, the analysis of protein profiles in inflammatory disease could be a useful tool for diagnosing the disease and predicting prognosis. The SPE pattern of dogs with AP revealed the characteristic features of acute phase response and analysis of APP concentration revealed increased concentrations of CRP, SAA, and haptoglobin. In particular, concentration of SAA in dogs with AP was significantly increased and showed positive correlation with that of CRP. These findings suggest that SAA could be a sensitive marker for inflammation in dogs with AP, similar to CRP. Future studies investigating SAAs in disease states of differing severities and responses to treatment will further increase our understanding of SAAs as diagnostic and prognostic markers in dogs with AP.

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