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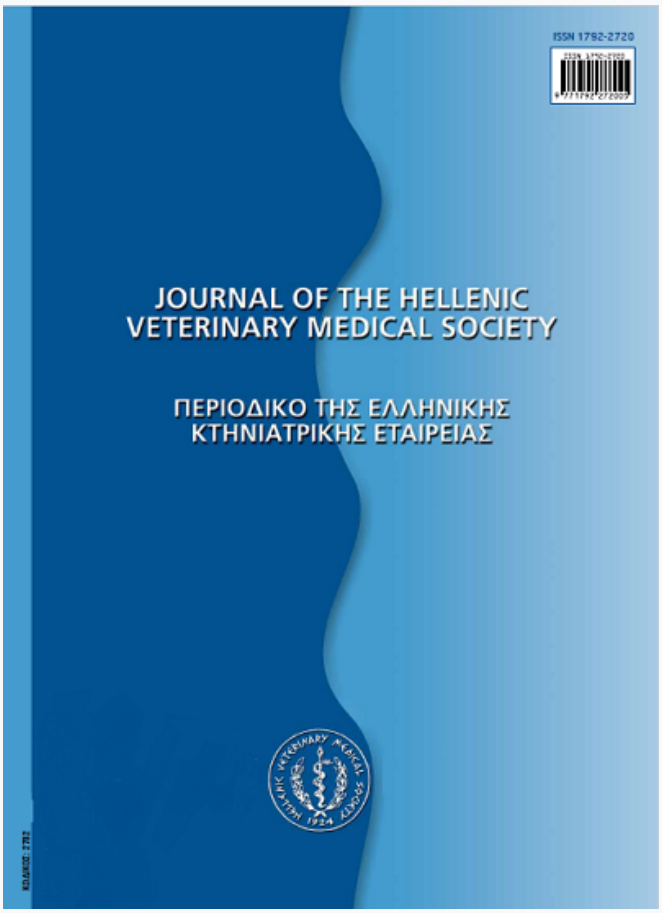

Acute phase proteins in diagnostics: more than expected

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Review article
Ανασκόπηση

Acute phase proteins in diagnostics: more than expected

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Πρωτεΐνες οξείας φάσης: νέα δεδομένα για τη διαγνωστική τους αξία στην κτηνιατρική εργαστηριακή διάγνωση

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ABSTRACT

The objective of this review is to provide updated information about how acute phase proteins (APPs) can be used in the process of diagnosis in veterinary medicine. For this purpose, recommendations about assay methodologies and basic principles regarding clinical application of APPs will be provided. In addition, the use of APPs for (1) the detection and quantification of inflammatory response, (2) the diagnosis of the underlying etiology and (3) the detection of selected non-inflammatory processes will be reviewed with practical examples.

Keywords: acute phase proteins, cat, diagnosis, dog

ΠΕΡΙΛΗΨΗ

Στην ανασκόπηση αυτή αναφέρονται τα νεότερα δεδομένα σχετικά με τη σημασία των πρωτεϊνών οξείας φάσης (ΠΟΦ) στη διαγνωστική διερεύνηση των νοσημάτων του σκύλου και της γάτας. Για το σκοπό αυτό γίνεται λεπτομερής αναφορά στη διαγνωστική αξία και την προτεινόμενη μεθοδολογία για τη μέτρηση των ΠΟΦ. Παράλληλα περιγράφεται με τη βοήθεια παραδειγμάτων από την κλινική πράξη η σημασία της μέτρησης των ΠΟΦ για τη διάγνωση και τη διαφορική διάγνωση των νοσημάτων του σκύλου και της γάτας.

Λέξεις ευρετηρίασης: γάτα, εργαστηριακή διάγνωση, πρωτεΐνες οξείας φάσης, σκύλος

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INTRODUCTION

Acute phase proteins (APPs) are serum proteins whose concentration changes in response to inflammation or stimulation of the immune system regardless of the inciting cause. APPs may either increase (positive APPs) or decrease (negative APPs) in the course of disease (Cerón et al., 2005). Positive APPs may be further classified into two groups:

- Major positive APPs, such as C-reactive protein (CRP) in the dog or serum amyloid A (SAA) in the cat. Usually they are virtually undetectable in the blood of healthy animals, but their concentration can increase between 10-1000 times upon stimulation.
- Moderate APPs, such as haptoglobin (Hp) or fibrinogen. They are present in the blood of healthy animals but may increase 2-10 fold in concentration following stimulation.

Major APPs show an early and high rise in concentration and a very rapid decline, whereas moderate APPs need more time to increase and return to normal values. An example of negative APPs is albumin that, since it is the most abundant protein in serum, decreases to promote the synthesis of other proteins related with inflammation.

APPs are generated in a fast reaction, that usually in the case of major APPs develops within hours (i.e., CRP showed significant increases 4 hours after a surgical trauma) and reaches peak concentrations in approximately 24 hours. This reaction starts as a sequela to any injury and is part of the innate immune response. In this sense APPs differ from immunoglobulins (Igs), which are involved in the acquired immune response, in two main aspects (Cerón, 2013):

- APPs are non-specific proteins since they are produced by any process that damages the organism, whereas Igs are specific against a selected pathogen.
- The production of APPs is very fast, appearing within hours, whereas in general Igs synthesis is evident at least after one-two weeks (seroconversion).

During the previous decade there has been a growing interest about the use of APPs in routine practice in companion animals, with their inclusion in routine biochemical profiles in several diagnostic laboratories all over the world. General guidelines have been published about the interpretation of APPs changes which contributed to their practical application (Cerón et al., 2008). Our experience after many years of studying these biomarkers, is that practitioners who regularly use APPs analysis tend to rely on these proteins as one of the most important biomarkers of inflammation and use them satisfactorily in many clinical situations for the diagnosis, treatment monitoring and predicting the outcome of inflammatory diseases. In this review we will try to focus on providing updated information and practical examples about how APPs can be used in the process of diagnosis. Although, due to their lack of specificity, APPs will have obvious limitations, there are a number of clinical situations in which APPs can provide valuable information in the diagnosis. In addition, since accurate measurements of APPs are crucial for an appropriate interpretation in diagnostics we will also deal with this topic in this review.

HOW TO MEASURE APPs

The acute phase protein tests may not be valid if run in a human laboratory or with human assays unless each assay is validated for the species being tested. The validation procedure should be repeated for each new batch of antiserum (Eckersall et al., 1999; Cerón et al., 2005). The dog and cat proteins have variable cross reactivities to antisera against human proteins, different reaction profiles and different reference ranges, thus some human assays are not suitable for use in dogs or cats (Fransson et al., 2007).

In addition it is recommended that working standards (purified protein or pools of acute phase serum) and control samples should be matched with the species under investigation (Eckersall, 1995). The use of specific species standards implies obtaining a similar affinity of antiserum against standards and samples, giving more accurate measurements, and also produces wider analytical ranges, avoiding the need for the dilution of samples (Tecles et al., 2007). Calibration of assay methods ideally should be har-

monized to ensure that results obtained in different laboratories are comparable universally and of consistent quality (Cerón et al., 2005). It is encouraging to note that very similar cut-off points for APPs in selected canine diseases have been established at different laboratories (Kocaturk et al., 2010; McClure et al., 2013).

Recently a series of point-of-care equipment for APPs measurements in veterinary medicine have been developed. These devices should be used only if adequately validated, and the need of having a special care should be stressed at this point. The authors have found that certain equipments do not have an adequate accuracy and precision in contradiction with published data or the "official" data that the manufacturer of the equipment provides.

Usually for most APPs, serum, EDTA or heparinised plasma (with exception of fibrinogen that needs plasma samples) can be used. It is important to know the effect of hemolysis, lipemia and bilirubinemia in the assay used for APPs measurement. APPs are fairly stable and the samples can be kept refrigerated for several days or frozen for long period preservation (Cerón et al., 2005).

BASIC ASPECTS FOR USE OF APPs IN DIAGNOSTICS

For diagnostic use it would be recommended to use a profile of APPs including at least:

- one major acute phase protein (APP) such as CRP in the dog or SAA in the cat. SAA can be also used in the dog, however the lack of automated or point-of-care analysers for SAA measurements in dog has limited its application. CRP does not function as a major APP in the cat.
- one moderate APP such as haptoglobin or fibrinogen.
- one negative APP such as albumin.

The reference values currently used in the authors' laboratory for the main APPs are:

-Canine C-reactive protein: <12 mg/L

-Feline serum amyloid A: < 1 mg/L

-Canine and feline haptoglobin: < 3g/L

-Fibrinogen:1-3 g/L

In inflammation there is an increase in both major and moderate APPs and a decrease in albumin (negative APP). It should be pointed out that in pregnant bitches an acute phase response with increases in CRP and Hp occurs at 21 days after fertilization, coinciding with embryonic implantation (Eckershall et al., 1993).

THE USE OF APPs IN THE DETECTION AND QUANTIFICATION OF INFLAMMATORY RESPONSE

Although APPs should be used and interpreted together with the total blood cell counts (CBC) data, they have several advantages compared to the measurement of white blood cell counts (WBC) to detect inflammation such as:

- APPs are more sensitive, especially in cases of mild or chronic inflammation. Moreover, in cases of severe and acute inflammation, such as babesiosis, the diagnostic sensitivity of CRP was significantly higher compared to WBC counts (Matijatko et al., 2007). Similarly, CRP was more useful than the WBC counts for assessing the severity of inflammation in different surgical procedures (Yamamoto et al., 1993).
- APPs are more stable than cells and measurements can be made in frozen serum samples.
- APPs detect inflammation in cases with suppression or decreased activity of the bone marrow.
- APPs quantify the magnitude of the inflammatory response in situations of severe inflammation where is a decrease in WBC.

This high sensitivity and fast stimulation of response allow APPs to *detect subclinical inflammation,* with changes in APPs occurring before the presence of clinical sigs. Therefore APPs are ideal for routine health checks, since an increase in APPs in an apparently healthy animal can indicate the presence of a subclinical disease or be predictive of an active disease developing in the near future. Suzuki et al. (2007) reported cases of dogs infected with *Babesia gibsoni* where high concentrations of CRP were documented despite the absence of obvious clinical signs or parasitemia. Also high values of CRP were also found in asymptomatic dogs infected by *Leishmania infantum* (Martinez-Subiela et al., 2002)

In addition to their use in detecting inflammation, APPs can be used for quantification of its magnitude. This can help to *evaluate the severity of the disease as well the presence of complications*. Some relevant examples include:

- Higher mean CRP and Hp values were found in dogs infected with *Ehrlichia canis* that had myelosuppression, compared to those with the uncomplicated disease (mean CRP and Hp values of 237.3 mg/l and 4.15 g/L, respectively, were documented in myelosuppressed compared to 144.2 mg/L and 1.96 g/L in the uncomplicated cases). This would probably reflect a more severe tissue damage and inflammatory reaction. Interestingly there were no significant differences in serum albumin. Therefore these positive APPs can be useful in assessing the clinical severity of canine monocytic ehrlichiosis, in association with other clinicopathological tests such as CBC or traditional serum biochemistry (Mylonakis et al., 2011).
- CRP has been proposed as a useful laboratory marker to assess the inflammatory process associated with inflammatory bowel disease (IBD) in dogs. Although CRP changes are not very pronounced and barely exceed the reference range established by many laboratories, increases in this APP (mean values of 15.33 mg/L) have been found in dogs with IBD that have active disease and overt gastrointestinal signs such as weight loss, vomiting, diarrhea, melena or tenesmus, and CRP values were strongly correlated with disease activity (Jergens et al., 2003).
- There is an evident acute phase response in dogs with generalized demodicosis, showing median values of CRP of 51.7 mg/L, which does not occur in the localized form. Measurements of CRP could therefore provide an aid to clinicians in deciding whether doubtful cases could be considered as generalized or localized, because values outside the reference range of the laboratory could be suggestive of the generalized form (Martinez Subiela et al., 2014).
- In mammary tumors, CRP showed major increases (mean values of 86.1 mg/L) in those with histologic evidence of malignancy and metastasis, compared to benign or non-metastatic malignant tumours (mean CRP values of 9.45 and 6.10 mg/L respectively) (Tecles et al., 2009). Therefore major increases of CRP in dogs with mammary tumours, in absence of evident inflammatory conditions, could be considered as an indicator of metastasis.
- APPs can be used to quantify inflammation associated with surgery. The magnitude of CRP increases in dogs subjected to surgery is generally related to the intensity of the surgical trauma. For example, in an study in which different surgical procedures were compared, CRP increase was more pronounced when severe tissue injury was inflicted in muscle or bone tissue by procedures such as orthopaedic surgery (Yamamoto et al., 1993).

APPs AS A DIAGNOSTIC AID IN THE ETIOLOGIC DIAGNOSIS OF INFLAMMATION

APPs are not useful in the direct etiologic diagnosis of inflammation but, *the magnitude of their increase can be informative* (Table 1). *Furthermore, it may help to reduce the list of diagnosis differentials in some situations as described below:*

- Marked increases of serum CRP concentration (higher than 100 mg/L) can raise the suspicion of systemic inflammatory response syndrome (SIRS) (associated with sepsis and a positive result of bacterial culture of appropriate samples) or an immune-mediated disorder. Some examples could be:

-In dogs with clinical signs compatible with respiratory diseases, bacterial pneumonia was identified with a specificity of 100% when CRP was higher than 100 mg/L, compared to other respiratory diseases/syndromes had significantly lower CRP values, such as bacterial tracheobronchitis (23 mg/L), chronic bronchitis (13 mg/L), eosinophilic bronchopneumonitis (5 mg/L), canine idiopathic pulmonary fibrosis (17 mg/L) and cardiogenic pulmonary edema (19 mg/L) (Viitanen et al., 2013).

-Dogs with autoimmune haemolytic anaemia had high CRP values early in the course of disease

Table 1. A guide for clinical interpretation of the magnitude of serum CRP increase in dogs

0-12	Normal
12-20	Slight increase of uncertain diagnostic value: *very mild inflammation *uncomplicated gastrointestinal disease *uncomplicated nasal disease
20-39	Increased levels: *mild inflammation * uncomplicated viral disease
40-100	Significantly increased levels: *moderate inflammation *generalized demodicosis *possible metastasis (mammary neoplasia)
>100	Severe inflammation: *septicemia *immune-mediated disorders: -immune-mediated hemolytic anemia (IMHA) -steroid response meningitis-arteritis (SRMA) -immune-mediated polyarthritis

(mean 191.2 mg/L) (Mitchell et al., 2009). Therefore, the concurrent presence of a severe anemia and elevated CRP values could be indicative of an immune-mediated mechanism for this anemia.

-Gastrointestinal disease usually produces very mild changes in CRP that does not exceed 21.5 mg/l (McCann et al., 2008). And in viral infections the acute-phase response is generally moderate or mild; for example, in human beings, CRP values of 20-39 mg/L have been reported for viral infections (Hjortdahl et al., 1991). Therefore, a major increase in CRP in a dog with parvovirus infection could indicate septicemia associated with the compromise of normal protective intestinal barriers (Kocaturk et al., 2010).

- In conditions where clinical signs could be produced by multiple inflammatory or non-inflammatory causes, the presence of a positive APP profile raises the suspicion for an infectious–inflammatory cause. For example:

- In cases of lameness, CRP can facilitate the differential diagnosis between immune-mediated polyarthritis that usually is characterized by major increases in this APP, and other conditions such as degenerative joint disease or intervertebral disk displacement that do not affect CRP values (Ohno et al., 2006).

-In dogs with compatible neurological signs, the presence of a marked increase in serum CRP (that usually is not accompanied by changes in CBC) may support the diagnosis of steroid responsive meningitis-arteritis (SRMA), versus other differential diagnoses such as meningoencephalitis, intervertebral disk disease, degenerative lumbosacral stenosis, central nervous system neoplasia or idiopathic epilepsy, that do not produce major increases in serum CRP (Bathen-Noethen et al., 2008).

-CRP together with the percentage of band neutrophils has been proposed as laboratory marker in order to differentiate pyometra and cystic endometrial hyperplasia/mucometra (CEH). CEH is not associated with bacterial infection, and a mucus material is accumulated in the uterus instead of the purulent material that appears in pyometra. A mean of 12.5 % band neutrophils versus 1.69 % and a CRP concentration of 200 versus 53 mg/L were found in pyometra versus CEH cases in a previous study (Fransson et al., 2004).

Nevertheless, in other situations such as nasal diseases, it seems that CRP does not help to differentiate the cause of the problem. Similar changes in CRP values have been noted in aspergillosis, non-specific rhinitis or neoplasia (Sheahan et al., 2010). In these cases CRP changes were not pronounced, so a marked elevation in this APPs in cases of nasal disease should prompt the clinician to look for concurrent or underlying inflammatory pathology that may be of clinical significance. In addition CRP does not appear to be useful for differentiating among causes of canine chronic gastrointestinal disease such as inflammatory bowel disease, parasitic infections, dietary and antibiotic responsive diarrhoea diarrhea, gastrointestinal neoplasia or presumed motility disorders (Mc Cann et al, 2008). Also, intestinal malabsorption caused by exocrine pancreatic insufficiency, wheat-sensitive enteropathy or anaerobic bacterial overgrowth did not produce increases in CRP in dogs (Caspi et al., 1987). So if there is an evident increase in CRP associated with any of these conditions it will indicate the presence of a complicating systemic inflammatory condition.

In cats SAA and AGP has been demonstrated to be a reliable aid in the diagnosis of FIP, because major increases in both APPs are associated with this condition (Duthie et al., 1997; Giordano et al., 2004). However, the concurrent increase of both APPs are not pathognomonic for this disease because it can be also found in other conditions such as septic processes or disseminated neoplasias (Hazuchova et al., 2012).

APPs AS A DIAGNOSTIC AID IN THE DETECTION OF NON-INFLAMMATORY PROCESSES

The divergence between the evolution patterns of major and moderate APPs could help to detect disease processes not associated with inflammation. The term "divergence" is used to describe the situation in which APPs of both groups do not change in a similar and proportional manner as should occur when there is an inflammatory stimulus. Some relevant examples are listed below:

- An increase in Hp concentration with normal CRP values in a dog with no history of glucocorticoid treatment could indicate the increase production of endogenous steroids, as in hyperadrenocorticism. This is due to the fact that glucocorticoids stimulate increases in haptoglobin and decreases in CRP (Caldin et al., 2009). This particular profile can potentially be used as an indication of canine hyperadrenocorticism. Also if in a dog diagnosed with hyperadrenocorticism there is an increase in CRP, it will imply the presence of a severe inflammatory stimulus that is able to overcome the inhibitory effect of steroids. Therefore the clinician should look for inflammatory complications such as severe sepsis, urinary infection, deep pyoderma, severe mastitis or immune-mediated haemolytic anemia (Caldin et al., 2009)
- A decrease in Hp with increased CRP could suggest haemolysis or internal haemorrhage leading to haemolysis. This is because one of the main functions of Hp is to link to haemoglobin upon its release from damaged erythrocytes to facilitate its degradation and avoid the oxidative stress caused by haemoglobin. For example, in 50 dogs naturally infected by *B. canis*, CRP concentration were found to increase (mean values of 170 mg/L) while haptoglobin remained within reference ranges (mean values of 2.7 g/L). These normal values of haptoglobin could reflect the mixed effect of inflammation, associated with moderate increases, and haemolysis which reduces the haptoglobin concentration (Matijatko et al., 2007).

CONCLUSIONS

Despite the limitations associated to their lack of specificity, APPs can be used in selected situations as a valuable diagnostic tool. It is expected that the information presented in this paper can be helpful for practitioners who are already using these biomarkers and further encourage others not familiar with their applications to use them for clinical diagnosis.

CONFLICT OF INTEREST STATEMENT

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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The authors would like to dedicate this work to all the Greek society for their sacrifice in the last years. In recognition for all their unique and invaluable past, present and surely future contributions in all the branches of arts, culture and science.

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