Review of Equine Acute-Phase Proteins

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Plasma concentrations of acute-phase proteins (APPs) increase during the acute-phase response to every process that leads to tissue damage (e.g., infections, trauma, surgery, and neoplasia). Measurements of APPs may be useful for diagnosing the presence of inflammation and tissue damage, prognostication, monitoring the effect of the therapy and the occurrence of post-operative complications or relapse, and monitoring herd health. APPs are potentially valuable adjuvants to clinical assessment of a patient. Author’s address: Department of Large Animal Sciences (Large Animal Surgery), Faculty of Life Sciences, University of Copenhagen, Dyrlægevej 48, DK-1870 Frederiksberg C, Copenhagen, Denmark; e-mail: stj@life.ku.dk. © 2007 AAEP.

1. Introduction
Monitoring the inflammatory response can be a clinical challenge, because signs of inflammation do not always manifest clinically. Under certain circumstances, blood-biochemical and/or hematological testing may be necessary to identify indiscernible inflammatory disease. Within the last couple of decades, interest has focused on the potential use of the acute phase proteins (APPs) as indicators of the presence, degree, and time course of inflammation, because these proteins are released in large quantities into the blood stream in response to infection and tissue injury. In humans, APP measurements are used for routine assessment of inflammatory status; however, in horses, the APP response has only been investigated to a limited degree, which precludes the use of APPs in evidence-based equine medicine. The purpose of this paper is to review what is currently known about the equine APPs and to discuss their possible applications in the equine clinic.

2. The Acute-Phase Response
The acute-phase response (APR) is a complex set of inflammatory reactions that occur after injury or infection. It is induced by all processes that lead to tissue damage (bacterial and viral infection, parasite infestation, trauma, surgery, ischemic necrosis, burns, and neoplasia). The APR is initiated when cells and tissues are injured. Injury elicits production of a large number of inflammatory mediators among which the cytokines play very important roles. The cytokines initiate the APR cascade through stimulation of several cell types. A central pathophysiological step of the APR is hepatic synthesis and as a result, increased plasma concentrations of APPs. Recent studies have shown that extrahepatic tissues can also be stimulated by cytokines to produce APPs.

3. The APPs
APPs are proteins that have serum concentration changes of at least 25% during the APR. Levels of
positive APPs increase during the APR. The group of positive APPs is further subdivided based on their pattern of response to stimulation. The major positive APPs have very low or undetectable levels in healthy individuals, and their serum concentrations increase 100 times during the APR (Table 1). The moderate or minor APPs are present in serum of healthy individuals, and their concentrations increase 1–10 times during the APR (Table 1). The negative APPs are proteins with plasma concentrations that decrease during the APR. In horses and many other species, albumin is a negative APP.7 During the APR, the demand for amino acids for synthesis of the positive APPs is markedly increased, which causes down-regulation of hepatic albumin synthesis and shunting of amino acids into synthesis of positive APPs.8

The different APPs have different kinetic profiles (Table 1). Some increase within a few hours of infection or tissue injury and reach peak values within 1–2 days, whereas others have a slower increase and remain elevated for longer periods of time. In horses, serum amyloid A (SAA) is an example of the former group.9,10 Haptoglobin, α1-glycoprotein (orosomucoid), and C-reactive protein all have intermediate response times.7,11–14 Fibrinogen and ceruloplasmin concentrations peak 7–10 days after an inflammatory stimulus and may remain elevated for several weeks.15–17

4. APPs in the Equine Clinic

The possible applications of APPs in the veterinary clinical chemistry have been comprehensively reviewed.18,19 However, the equine APP response has not been investigated thoroughly, and these reviews mainly concern species other than the horse. Use of fibrinogen and SAA for detection of inflammation specifically in horses has been reviewed by Andrews et al.20 and by Jacobsen and Andersen.21

Cytokines inducing hepatic APP synthesis are released from inflamed or injured tissue independent of the cause of the tissue injury. As a consequence of this, APPs are non-specific markers of inflammation and cannot be used for making etiological diagnoses. Therefore, measurements of APPs are mainly relevant for the following purposes: diagnosing the presence of inflammation, prognostication, monitoring the effect of the therapy and the occurrence of post-operative complications or relapse, and monitoring herd health.

Diagnosing

In horses, APP levels have been reported to be elevated during bacterial and viral infections (such as infectious arthritis, strangles, pneumonia, sepsis, enteritis, and herpes and influenza virus infection), during parasite infestations, after surgery, after parturition, and when colic, abscesses, laminitis, or grass sickness are present.7,9–17,22–31 Because of its role in clearance of free iron and hemoglobin from the circulation, haptoglobin concentrations decrease in horses with intravascular and extravascular hemolytic anemia. Serum haptoglobin has been reported to be an early and sensitive indicator of hemolysis and is useful for diagnosing and prognosticating anemias without clear pathogenesis.32

The diagnostics potential of APPs may be particularly relevant for differentiating between infectious and non-infectious diseases that cause weakness in neonatal foals. In this type of patient, clinical signs are often non-specific, and diseases in neonates are, therefore, notoriously difficult to diagnose. Remarkably high plasma levels of SAA have been detected in foals with sepsis, but SAA levels...
are also increased in foals suffering from local infections.\textsuperscript{23,33,34} Serum concentrations of SAA in foals with non-infectious causes of weakness (e.g., prematurity, failure of passive transfer, neonatal maladjustment syndrome, isoerythrolysis, and meconium colic) have been reported to be in the normal range\textsuperscript{23,34} or slightly elevated.\textsuperscript{33} Stoneham et al.\textsuperscript{34} suggested that a cut-off value of 100 mg/l could be used to differentiate infectious from non-infectious cases and that inclusion of SAA in sepsis-scoring systems might improve diagnosing of neonatal sepsis and as a result, the chance of successful treatment. Hultén and Demmers\textsuperscript{35} showed that SAA was much more accurate in distinguishing infectious from non-infectious diseases than plasma-fibrinogen concentrations and leukocyte counts, whereas a study on Rhodococcus equi infection showed that total leukocyte count had significantly higher diagnostic performance than fibrinogen, which had low sensitivity and specificity in early identification of infected foals.\textsuperscript{35}

Prognostication

The magnitude and duration of the APP response have been shown to reflect the severity of infection and to correlate with the amount of underlying tissue damage and inflammation in cattle and humans.\textsuperscript{36,37} Therefore, APP levels may serve as indicators of prognosis. In horses, it has been shown that intra-articular injection of 3 µg of lipopolysaccharide elicits a larger SAA response than injection of 1 µg, which suggests that APP concentrations reflect the extent of the underlying inflammation in the horse.\textsuperscript{38}

In humans and dogs, levels of SAA and C-reactive protein have been used for prediction of outcome and post-operative complications during neoplastic disease, surgery, and burns and for diagnosing relapse during immune-mediated arthritis.\textsuperscript{39–41} In sheep with dystocia, high haptoglobin levels have been shown to indicate dead lambs in utero and consequently, increased surgical risk and poor prognosis.\textsuperscript{42} A recent study\textsuperscript{31} of horses hospitalized with colic showed that SAA levels at admission were higher in non-survivors than in survivors. Although this finding suggests that SAA measurements may be used for prediction of prognosis, concentrations differed only slightly between non-survivors and survivors (median concentrations in the two groups were 10.8 and 1.4 mg/l, respectively), and Vandenplas et al.\textsuperscript{31} concluded that sensitivity and specificity did not seem to be high enough to be clinically useful. In a study on critically ill and septic foals, high fibrinogen concentration was among the most significant predictors of mortality.\textsuperscript{43} APP measurements might become a valuable tool for veterinarians in advising horse owners on treatment of their animals.

Monitoring

The major equine APP SAA seems particularly suited for real-time monitoring of inflammatory activity.\textsuperscript{21} The low or undetectable SAA levels in healthy horses facilitate interpretation of mildly elevated concentrations, and the quick response time and short half-life of SAA cause its blood levels to increase quickly after an inflammatory stimulus has occurred\textsuperscript{38} and to decrease in close parallel with successful treatment and resolution of disease.\textsuperscript{9,23} This is in contrast to fibrinogen and haptoglobin, which are less suited for monitoring of the disease activity. Fibrinogen and haptoglobin are present in high levels in blood of healthy horses, and their amplitude of response is much lower than that of SAA (Table 1). Moreover, it takes days for their levels to increase after an inflammatory stimulus, and concentrations stay elevated for an extended period of time after the disease has been resolved.\textsuperscript{7,32} Certain pathological conditions (diseases characterized by hemolysis, disseminated intravascular coagulation, or changes in vascular permeability) may cause levels of haptoglobin and fibrinogen to decrease,\textsuperscript{20,32} thus masking increases induced by inflammatory processes.

Serum amyloid A seems to be able to monitor response to treatment of infections in the respiratory tract. A study on Rhodococcus equi pneumonia showed that foals had elevated plasma SAA and fibrinogen levels on admission to the hospital. Whereas SAA levels decreased with successful treatment and clinical improvement, fibrinogen levels were still high on discharge.\textsuperscript{23} APP levels may also be an aid in determining when a horse can return to training after having recovered from an airway infection. In horses with a particularly severe influenza-virus infection or in horses suspected of having contracted secondary bacterial infection, SAA levels remained elevated for an extended period of time compared with horses with a milder course of infection.\textsuperscript{25}

Surgery has been shown to induce an APR and cause elevated plasma levels of SAA, fibrinogen, haptoglobin, α1-acid glycoprotein, and ceruloplasmin.\textsuperscript{11,12,14,15,26,30,44} Two small studies (each with only two horses) suggested that general anesthesia alone had no effect on SAA and fibrinogen levels.\textsuperscript{7,10} The normal post-operative APP response has a rise-and-fall pattern, and sustained increases in APP levels post-operatively may indicate post-operative complications. In castrated horses, high SAA concentrations 8 days post-surgery suggested that surgery had been complicated by excessive inflammation/infection.\textsuperscript{26}

Herd Health

Measurements of APPs in large groups of horses could be useful for several purposes. Serum amyloid A measurements may be used to monitor spread of infections through farms or stables; levels remained undetectable in horses, which (despite being in contact with infections such as herpes virus or...
strangles) did not contract the disease.\textsuperscript{10} This could be useful when setting up quarantines.

APPs have been suggested to be sensitive markers of subclinical disease in cattle.\textsuperscript{45} This has not been investigated in horses. In this context, APP measurements might be useful for routine monitoring of health status in race and performance horses, thus avoiding that subclinically ill horses are subjected to the stress of transportation, competitions, and races. Detection of subclinical disorders could serve as a basis for advice to trainers by providing a management tool for adjustments of training intensity that would potentially decrease the risk of horses breaking down.

It is not yet clear if routine APP measurements can be used for detection of disease in horse herds. A study on \textit{Rhodococcus equi} pneumonia in foals showed that SAA measurements in bimonthly blood samples could not be used as a screening test for the early detection of pneumonia,\textsuperscript{46} but it was not clear if the low sensitivity resulted from long sampling intervals, misclassification of infection status, or \textit{Rhodococcus equi}-infected foals having too small of a SAA response.

5. Extrahepatic Production of APPs

In horses as well as other species, SAA synthesis has been shown to take place in several cell types other than hepatocytes during acute-phase states.\textsuperscript{3–5} This extrahepatic synthesis occurs particularly in endothelial cells and epithelia of organs communicating with the external environment (e.g., the gastrointestinal tract, the mammary gland, and the airways), which may suggest that SAA plays a role in host-defense mechanisms and local protection against invading microorganisms. In horses, SAA is synthesized locally in the mammary gland and in joints, and the protein has been shown in normal colostrum and synovial fluid from horses with experimentally induced arthritis.\textsuperscript{4,27} It has been shown that SAA levels in synovial fluid can help distinguish infectious from non-infectious (with less inflammation) joint disease, and when synovial fluid SAA levels were measured sequentially in the same patient, levels reflected effect of treatment.\textsuperscript{27}

Measuring local APP levels improves precision of diagnosis, because it provides information on inflammatory/infectious status of the particular organ of interest. However, this potential has been explored only to a very limited degree, and much more research is needed in this field.

6. Measuring Equine APPs

Several methods for measuring equine APPs have been developed, including enzyme-linked immunosorbent assay for SAA,\textsuperscript{44,47} slide reversed passive latex agglutination test for SAA,\textsuperscript{48} single radial immunodiffusion for SAA, haptoglobin, C-reactive protein, α1-acid glycoprotein and ceruloplasmin,\textsuperscript{9,11,12,14,15} electroimmunoassay for SAA,\textsuperscript{10,35} hemoglobin-binding capacity assays for haptoglobin,\textsuperscript{20} and latex agglutination immunoturbidimetric assay for SAA and haptoglobin.\textsuperscript{34,50,51} Haptoglobin and SAA assays developed for use in multiple species including the horse are commercially available,\textsuperscript{a} but equine validation studies for these assays are not yet available. The above mentioned assays are mainly relevant for research purposes, but recently, a test system developed for use in equine practice and small diagnostic laboratories has become available in Scandinavia and the United Kingdom.\textsuperscript{b} This system allows serum concentrations of SAA and haptoglobin to be measured within 30 min, and preliminary data show that concentrations are measured accurately and precisely.\textsuperscript{c} A human SAA assay\textsuperscript{d} for use in larger diagnostic laboratories has been validated in the horse\textsuperscript{51} and is commercially available in the United Kingdom.

Several assays are available for assessment of fibrinogen. It may be assessed with sufficient precision by heat-precipitation methods. These are commonly used methods in the equine practice, because they are quick and do not require specialized equipment.\textsuperscript{20} Also, the glutaraldehyde test\textsuperscript{e} can be used for measuring combined levels of globulins and fibrinogen semiquantitatively. Glutaraldehyde binds free amino groups in fibrinogens and immunoglobulin and as a result, creates a clot; the clotting time of the test estimates the concentrations of inflammatory proteins. However, a study by Brink et al.\textsuperscript{32} showed that the test was only moderately able to differentiate acute and chronic inflammation in horses and that clotting time was correlated to globulin but not to fibrinogen levels. Before further studies are available, this test should be used cautiously.

It is important to keep in mind that APP concentrations measured by different assays are not directly comparable, and therefore, absolute concentrations and cut-off values may differ between different studies. Also, age, gender, and physiological condition of the horse should be considered when interpreting a measured APP concentration. Small and seemingly unimportant, yet statistically significant, differences in serum concentrations of C-reactive protein, haptoglobin, ceruloplasmin, and α1-acid glycoprotein have been shown between different age groups, between males and females, and between pregnant and non-pregnant mares.\textsuperscript{11,12,14,15} Most studies show no differences in SAA and haptoglobin levels between neonatal foals and older horses.\textsuperscript{30,33,34}

References and Footnotes


*p*Phase Haptoglobin and Phase SAA, Tri-Delta Diagnostics, Morris Plains, NJ 07950.

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