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Testing for inflammation: Focus on acute phase proteins

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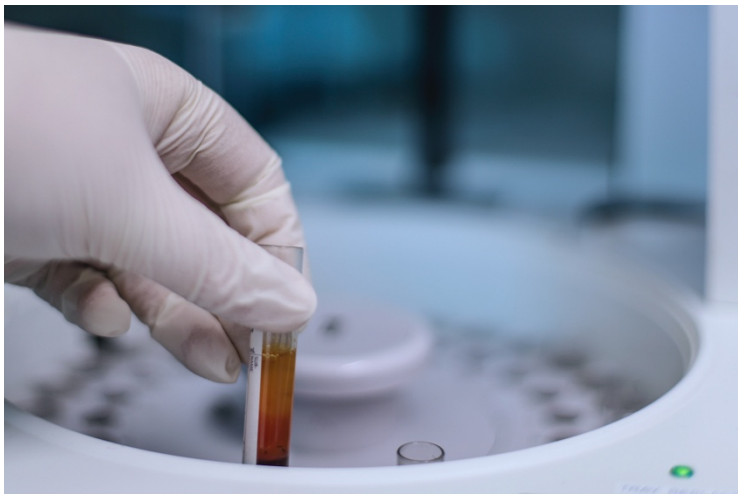
Acute phase proteins (APPs) are a diverse group of proteins produced in the liver as part of the acute phase response. The acute-phase response (APR) is a set of inflammatory reactions that occur after tissue injury or infection. It is activated by any process that causes damage to tissues and cells such as bacterial and viral infection, surgery, trauma, parasite infestation and neoplasia (cancer), among others. This tissue injury triggers a complex pathway which stimulates the liver to increase production of the acute phase proteins. Many acute phase proteins can be measured, although only fibrinogen, serum amyloid A (SAA), albumin, and globulin are widely used in equine practice. Other acute phase proteins are commonly used in human clinical pathology or in small animal medicine but are either less helpful or have been the subject of less investigation in horses.

Acute phase proteins may indicate underlying subclinical disease leading to poor performance. However, elevations in APPs are not specific for a certain disease process and can increase with inflammation in the absence of infection. Acute phase proteins should always be used in conjunction with clinical examination findings and other diagnostic tests to identify the cause of the inflammatory response. Inflammation does not always manifest with clinical signs, and acute phase proteins may be useful for determining the need for further investigation or for guiding the endpoint of treatment.

Serum Amyloid A (SAA)



Major APPs have concentrations that are low or clinically undetectable in normal animals but rapidly increase greater than 10-fold during the APR and rapidly decrease with disease resolution. SAA is the only major acute phase protein in the horse, is commonly used in practice and has been widely studied. SAA is a more sensitive marker of inflammation than other commonly evaluated laboratory parameters such as fibrinogen and white blood cell count. The increase in SAA occurs sooner and is of greater magnitude than that of fibrinogen. SAA concentrations increase up to 1000 times as soon as 6 hours after stimulation and, once the inflammatory trigger is removed, concentrations decrease within 12 hours (Long *et al*., 2020).

SAA is produced in the liver and the blood concentration is very low in healthy horses (<10ug/L). The rapidity of increase and decrease in SAA means that it is useful as a monitoring tool to help assess response to treatment or resolution of disease. The availability of stable side SAA monitors removes the delay of waiting for blood test results, although more complete blood tests will offer additional useful information in most situations. It is important to use the same machine and the same type of blood

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tube for monitoring SAA, as discrepancies in the absolute value may occur between lab-based and stable-side analysers.

SAA is non-specific and increases in many situations, including following routine surgery or vaccination. It does not appear to be increased in horses with gastric ulceration (Spanton *et al.*, 2020). SAA was a more reliable indicator of early inflammation than elevated rectal temperature in horses travelling long distance by air (Oertly *et al.*, 2021). Flat racing and strenuous training have been shown to cause very mild increases in SAA which return to baseline in 7 days (Turlo *et al.*, 2015). A weak correlation between SAA concentration and days in training was seen in UK Thoroughbreds in one study (Mack, 2014). Overall, SAA concentrations appear to increase to a greater degree with endurance exercise compared to short-distance work, including fast work (Long, 2020). More research is needed to identify patterns in healthy racehorses in training to make SAA more useful as a monitoring tool.

Fibrinogen

Fibrinogen aids with normal blood clotting and is always present in the blood of healthy horses (<4 g/L). Fibrinogen is a moderate acute phase protein with concentrations increasing 1-10 time over a 24–72-hour period in response to an inflammatory process. This slow increase means that it is less suitable than SAA for detecting very acute/recent inflammation. It also takes longer to return to normal concentration and may remain elevated after inflammation has resolved. Very high concentrations of fibrinogen (>7 g/L) are often seen in cases of internal abscessation.

Characteristics of Serum Amyloid A and Fibrinogen	
SAA (major APP)	**Fibrinogen (moderate APP)**
• Low or undetectable in healthy horses	• Always present in plasma in healthy horses
• Increases >10 fold rapidly during acute inflammation	• Increases 1-10X in response to inflammation
• Rapid decrease with disease resolution	• Takes days to weeks to peak and to return to baseline
• Relapse or secondary infection results in increases	
From: Crisman *et al.*, 2008; Long *et al.*, 2020; McGovern, 2015	

Albumin

Albumin is one of the main proteins in the blood, along with globulin. It is a negative acute phase protein, which means that the concentration decreases in response to inflammation. Chronic inflammation (over days to weeks) tends to cause this decrease. Albumin is therefore not useful as a marker of acute inflammation. Other causes of decreased albumin are very common, and the clinical picture needs to be considered. Low albumin concentration is seen commonly with increased globulin concentration in inflammatory conditions.

Globulin

An increase in globulin concentration is characteristic of a chronic inflammatory condition and is less useful for acute inflammation. Serum protein electrophoresis can be run to determine which subset of globulins are increased, although this is often unrewarding as multiple fractions are typically increased. Increased production of a single immunoglobulin is called a monoclonal gammopathy and is usually the result of neoplasia.

C-reactive protein

C-reactive protein (CRP) is widely used and has been extensively studied in people and in dogs, where it is a major APP. Increased CRP has been associated with castration, enteritis, pneumonia, and arthritis in horses (Takiguchi *et al* 1990). However, CRP is considered a minor APP in the horse. This means that it does not increase as much as SAA in the APR, making it less useful for monitoring inflammation. As such, more recent research has focused on SAA over CRP and information regarding CRP in the horse is lacking.

Other acute phase proteins

There are many other APPs which have been evaluated in horses in a limited number of studies. Procalcitonin has been shown to increase in horses affected by systemic inflammatory response syndrome (SIRS) when compared to normal horses (Bonelli *et al* 2015). Ceruloplasmin is known to increase with sterile inflammation and surgical inflammation (Johns 2014). α1 acid glycoprotein is likely to be associated with chronic rather than acute conditions (Crisman *et al*., 2008) but data in the horse is very limited.

Iron

Iron is not considered an APP, as it is a mineral rather than a protein. However, iron is a useful marker of inflammation. Unlike fibrinogen and SAA, blood iron concentration rapidly decreases in response to inflammation, as it becomes sequestered in the cells. It has been shown that iron decreases as soon as 5 hours after induction of inflammation in rats (Hershko *et al.,* 1974). A study in hospitalised horses found that iron was better than fibrinogen for detecting acute inflammation (Borges *et al*., 2007). A continued decrease in iron concentration and increase in fibrinogen concentration with hospitalisation was associated with a poor prognosis.

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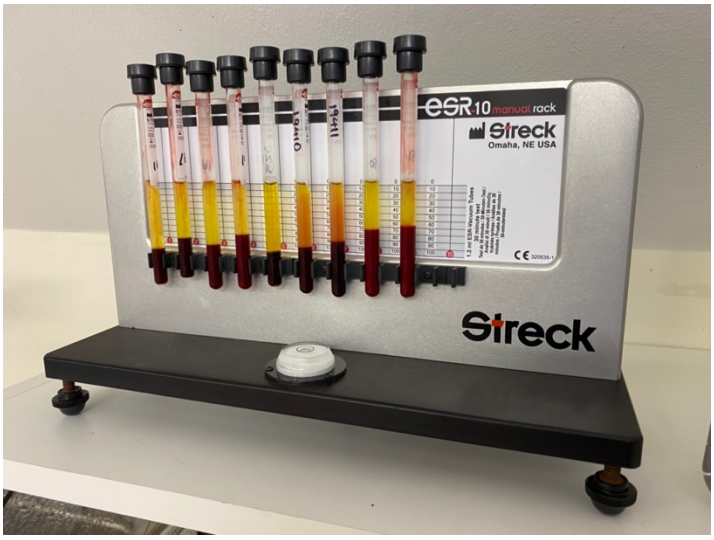
Iron can be used alongside SAA and fibrinogen to give a more complete picture of the acute inflammatory response.

Erythrocyte sedimentation rate (ESR)

The erythrocyte sedimentation rate (ESR) is the rate at which red blood cells in anticoagulated whole blood descend in a specific tube over a period of one hour. It is a non-specific measure of inflammation. Increases in ESR are caused by inflammation or anaemia. Unlike other species, healthy equine red blood cells often stack on top of each other (rouleaux formation), which can increase the ESR in the absence of inflammation (Latimer, 2012). Changes in temperature were also shown to affect the ESR in a subset of racehorses (Dintenfass and Fu-Lung, 1982). The ESR was historically popular in horses when measurement of acute phase proteins was not widely available but has been superseded by more accurate markers of inflammation such as SAA.



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