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Serum amyloid A in equine health and disease

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Summary

Serum amyloid A (SAA) is the major acute phase protein in horses. It is produced during the acute phase response (APR), a nonspecific systemic reaction to any type of tissue injury. In the blood of healthy horses, SAA concentration is very low, but it increases dramatically with inflammation. Due to the short half-life of SAA, changes in its concentration in blood closely reflect the onset of inflammation and, therefore, measurement of SAA useful in the diagnosis and monitoring of disease and response to treatment. Increases in SAA concentration have been described in equine digestive, reproductive and respiratory diseases and following surgical procedures. Moreover, SAA has proven useful for detection of some subclinical pathologies that can disturb training and competing in equine athletes. Increasing availability of diagnostic tests for both laboratory and field use adds to SAA's applicability as a reliable indicator of horses' health status. This review article presents the current information on changes in SAA concentrations in the blood of healthy and diseased horses, focussing on clinical application of this biomarker.

Keywords: horse; SAA; inflammatory diseases; acute phase reaction; acute phase proteins

Introduction

Monitoring the onset of inflammatory responses, although nonspecific, can give clinically important information, particularly when clinical signs are vague or hard to differentiate. Tests suitable for such monitoring should mirror the onset of inflammation and should be easily measured in blood. Due to the complicated nature of the inflammatory processes and the wide variety of humoral factors involved, many investigations have been focused on selecting appropriate biomarkers. Currently, it is believed that proteins produced during acute phase response (APR) meet these criteria.

The APR is an early, nonspecific, systemic reaction induced by tissue injuries of various types, including inflammation, infection, trauma or any other disturbances of homeostasis [1]. The APR is triggered by pro-inflammatory cytokines, which promote local and systemic effects, including acute phase proteins (APPs) synthesised in the liver [2]. The concentrations of APPs in blood change dramatically, reflecting the onset of APR but the pattern of these changes is species specific. Serum amyloid A (SAA) is the major acute phase protein in horses, recognised as the most sensitive indicator of the APR in this species [3]. Its concentration increases as early as 6 h after stimulation with infection or tissue injury [4,5] and within 24–48 h it can be up to 1000-fold of the initial concentration [6]. When inflammation resolves, SAA concentration drops within 12 h [4].

SAA is a 9–11 kilodalton apoprotein [7], which circulates in complex with high-density lipoproteins also produced in hepatocytes. In horses, several extrahepatic isoforms of SAA, synthesised mainly in endothelial cells and epithelia of the organs in contact with the external environment (e.g. the gastrointestinal tract, mammary gland and airways), have been identified [4,8]. It is secreted into the colostrum by mammary gland epithelial cells, but also into the synovial fluid by articular chondrocytes and by endometrium, which may indicate SAA participation in the shaping of local immune response [9,10].

SAA concentrations in the blood of healthy horses have generally been reported to range from <0.5 to 20 mg/L [4,5,11,12]. This wide range reflects individual features of the immune response and probably also the methods of measurement. Currently, the immunoturbidimetric assay is believed the most accurate, however, ELISA tests and "near patient testing kits" are widely used. Therefore, the best way to interpret the SAA concentration is to use an individual normal value, measured when the animal is healthy, to be compared with future diagnostic results. This allows straightforward interpretation of moderate increases in SAA, which have been considered more sensitive than the total white blood cell counts [13], C-reactive protein and fibrinogen [1].

The precise roles of SAA are not fully understood. Several functions have been considered on the basis of its in vitro effects (Fig 1). It is believed to contribute to inhibition of lymphocyte proliferation, platelet aggregation, phagocytosis and also stimulation of monocytes and neutrophils migration, prostaglandin synthesis and metalloproteinases activation [14]. Increases in SAA concentrations have been found in experimentally induced arthritis [15], respiratory diseases [8,16,17], colic [18,19], diarrhoea in foals [17], following surgery [20], parturition [5], vaccination [21], after strenuous exertion [22] and transportation [23].

SAA concentrations in healthy horses

In healthy horses, various concentrations of SAA have been reported (Supplementary Item 1) but generally concentrations are towards the lower or middle level of the commonly accepted reference range (0–20 mg/L). There are no sex-related differences [8,17], however, an age-related effect has been reported [5]. In healthy neonatal foals (<1-week-old) median SAA concentrations were 0.9, 4.0 and 2.5 mg/L on the 1st, 2nd and 3rd day of life respectively [24]. A weak positive correlation between SAA concentration and age was reported in 2- to 10-year-old Standardbreds, [25] but no age effect was demonstrated in a large group of Huculs [26].

Physiological changes in SAA concentration have been reported in pregnancy. In pregnant mares, SAA concentration remained stable within the normal range during the 4 months before parturition but increased from 1 week before until 1 month after foaling [5], which may result from tissue damage during the displacement of the fetus. Significant increases in SAA concentrations were noted at 12 h (0.7–305 mg/L) and 36 h (0–1615 mg/L) and returned to basal concentrations within 60 h post-partum [6]. Other studies have shown no changes in SAA concentrations after parturition in mares carrying normal pregnancies and delivering normal foals, and after insemination with frozen–thawed semen [27,28].

SAA concentrations and exercise

In horses, an exercise-induced APR has been described after long distance (120 and 160 km) endurance rides, strenuous training and racing [13,22,29–31]. In endurance horses, the exercise-induced APR is characterised by a marked increase in SAA but not haptoglobin and CRP

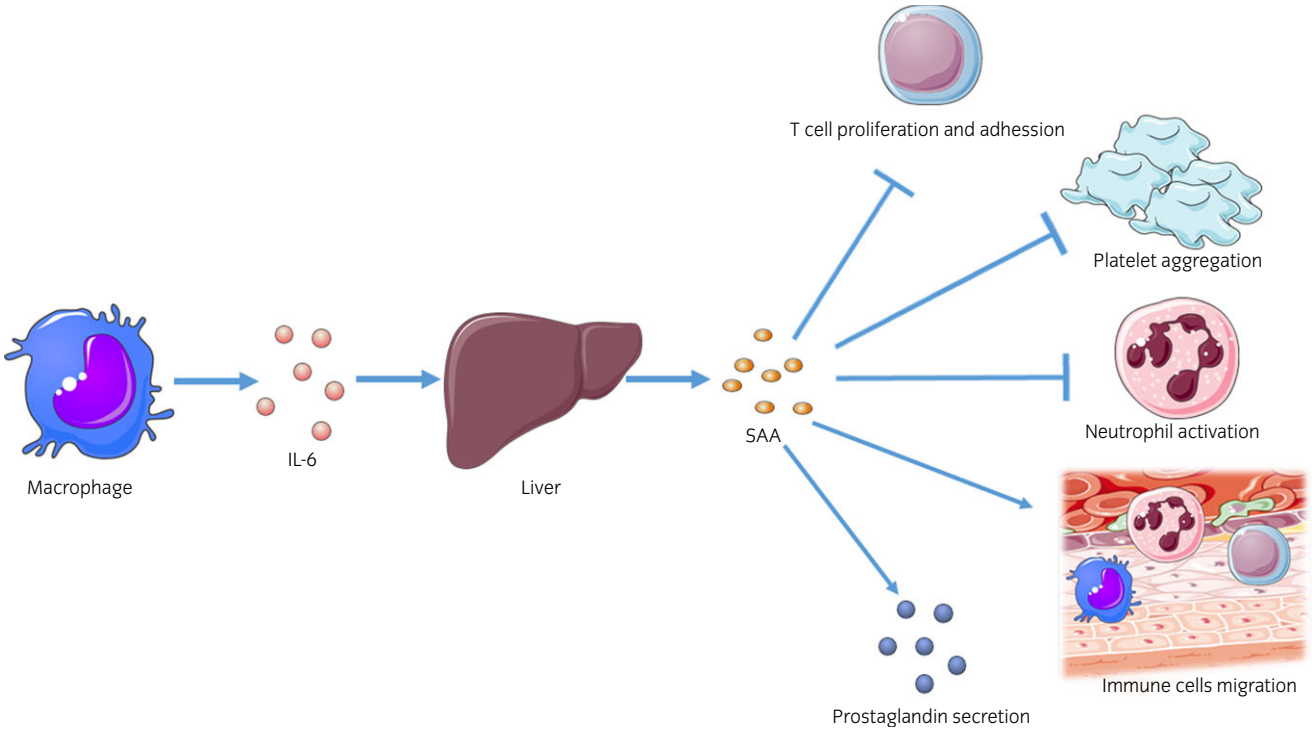

Fig 1: Serum amyloid A (SAA) as a multifunctional protein in modulating the inflammatory response. IL-6 produced by macrophages after local stimulation promotes the hepatic production of SAA which has roles in inhibition of lymphocyte proliferation, neutrophil activities, adhesion of T lymphocytes to extracellular matrix, platelet aggregation and induction of adhesion, migration and tissue infiltration of monocytes, neutrophils and prostaglandin synthesis (This figure was prepared using Servier Medical Art. https://smart.servier.com/).

concentrations [22]. In horses that completed long-distance rides and successfully passed veterinary inspection, SAA concentrations increased more than 10-fold [28], whereas after moderate-distance rides or strenuous training in inexperienced horses, a 2–4-fold increase in SAA concentration was observed [22,30]. Flat racing induced an approximately 7-fold increase which returned to the baseline concentration within 2 days [31]. In all studies of the impact of exercise on SAA, the highest individual SAA concentrations were still relatively low compared with those reported in horses with acute inflammatory diseases. In endurance horses, precompetition SAA concentrations may be an aid in the evaluation of general health and training status [29,30]. Interestingly, endurance horses, prepared for the longest distances (120–160 km), did not complete the competitions when their SAA concentration before the ride exceeded 1 mg/L [29]. However, in that study the sample size was small and more work is required to determine an appropriate discriminating cut-off value.

In racing Thoroughbreds, median SAA concentrations 3–4 days after racing were significantly higher in individuals with orthopaedic injury, SAA being a better indicator of inflammatory condition than leukocyte count [13]. In eventing horses at the end of competition, a twofold increase in SAA concentrations has been demonstrated [32]. No correlation between the concentrations of SAA and poor performance was detected in serially sampled individuals [33]. SAA monitoring during training may become a useful criterion for assessing general health of the horse and therefore contribute to evaluation of their prospective performance, but more investigations are necessary to support interpretation of the patterns of changes seen.

SAA in foal diseases

In clinical practice, the concentration of SAA may be helpful in early stage detection of infectious diseases, although they still must be considered in conjunction with a detailed history, physical examination, haematology and other ancillary diagnostic tests. An increase in SAA concentration is nonspecific, so does not indicate particular disease, but may prompt rapid therapy which is particularly important in the management of newborns. Sepsis is an important cause of mortality in the neonatal period. Signs of sepsis are nonspecific and may be subtle thus early detection poses a diagnostic challenge, but together with appropriate therapy is critical for case outcome [17,24]. Bacteraemia, septic arthritis and bacterial pneumonia produce high concentrations of SAA in foals [17,24,34,35]. In contrast, noninfectious causes of neonatal weakness have been characterised by normal to only slightly increased SAA concentrations [24,34]. Also foals with umbilical abscesses had low SAA concentration, which can be explained by the isolated character of inflammation. Thus, localised infection may be accompanied by normal SAA concentration. Increased SAA concentrations strongly suggest the possibility of systemic infection, but should never be interpreted as an indication for antimicrobial therapy. Detection of high SAA concentration warrants further investigation of infection, but the use of antimicrobials should be based on identification and susceptibility testing of the target pathogen(s) or, if this is not possible, should be based on epidemiological information and local knowledge of patterns of infection and antimicrobial susceptibility of the target bacteria. Clearly, national and regional antimicrobial policies should also be considered.

A cut-off value of 100 mg/L for general differentiation between infectious and noninfectious cases has been proposed [24]. However, in foals with bronchopneumonia on farms with endemic *Rhodococcus equi* infections, SAA does not necessarily increase. SAA concentrations below 5 mg/L were reported in 15 of 54 (28%) pneumonic foals and in foals between 3 weeks and 5 months, and SAA has low sensitivity and specificity for detecting *R. equi* pneumonia [36]. In the foals from *R. equi* endemic farms, SAA concentrations neither correlated with radiographic score and severity of pulmonary lesions nor ultrasonographic evidence of pneumonia, [36] and weekly testing SAA concentration was not an accurate marker of infection [37,38]. Reasons for failure to detect increased SAA concentrations in individuals with pneumonia and inflammation remain unknown but this may relate to the chronicity of disease [36]. Also, concurrent but unrelated

infection and errors in differentiation between healthy and subclinically infected foals might affect the study results [36].

Reproductive disorders

Female reproductive disorders are common and often lead to infertility in broodmares. Commonly used tools for assessing uterine health include clinical examination, cytology, bacteriology and histopathology; however, results may be inconclusive even when the combination of all methods is used [39,40]. SAA has been shown to rise significantly (11.36–22.90 mg/L) in Arabian mares with pyometra and has been suggested as a useful biomarker for this condition together with cardiac troponin I and pro-inflammatory cytokines [39]. In experimental endometritis, induced by intrauterine infusion of *Escherichia coli*, the changes in SAA concentrations varied according to the dose of the pathogen. A high (10^9 CFU) dose produced 10- to 100-fold increases in both blood and endometrial SAA concentrations, while administration of a low (10^5 CFU) dose resulted only in an endometrial response, manifested as the increase in mRNA expression of SAA in endometrial tissue as early as 3 h post-inoculation [9,41]. However, SAA concentration has failed as a marker of naturally occurring subclinical endometritis. In Icelandic mares with a history of infertility, SAA concentrations varied widely, in nine mares it reached very high concentrations (even 595.71 mg/L) but SAA concentrations were not associated with subclinical endometritis [40].

Infectious and noninfectious endometrial inflammations are the commonest causes of Early Embryonic Death (EED), occurring during the first 40 days of pregnancy. In EED mares, more than 20-fold increases in SAA concentration have been reported [42]. Placentitis leads to abortion or the birth of premature and septic foals. In experimental models of placentitis, SAA concentrations rapidly increased and remained high until abortion [6,27]. In ascending placentitis, experimentally induced by intracervical inoculation with *Streptococcus equi* spp. *Zooepidemicus*, SAA concentration increased up to 100-fold within 2–4 d.p.i. and abortion occurred within 5–25 days [27]. In another experimental study, SAA concentration rose at a similar time post-inoculation and vulvar discharge was the first clinical sign [6]. In naturally occurring placentitis, the onset is usually more insidious and vaginal discharge is not usually the first sign [43]. Thus, these experimental models may represent superacute inflammation [6]. The amount of organisms inoculated is not standardised across studies [6,27] and care must be taken in extrapolating observations made in experimental models to the naturally occurring condition. Possibly, if the process was not superacute but gradual, allowing for diagnosis and treatment before irreversible damage of placenta and the fetus, SAA may have increased prior to any clinical appearance [6]. Measurement of SAA in fetal heart blood may also help in understanding the nature of abortion. Erol *et al.* reported that SAA concentrations in fetal blood were significantly higher, reaching from 10.5 to 40 mg/L in the presence of infectious and/or inflammatory process in feto-placental tissues [44]. However, low SAA concentration resulting from degradation may occur in fetuses that have died in utero a long time before the abortion due to chronic placentitis without a systemic infection. Therefore, measuring the concentrations of SAA may help to detect mares with placentitis and at risk for abortion. Moreover, the administration of antibiotics and anti-inflammatory drugs in mares with placentitis successfully prevented the rise of SAA, thus, SAA concentration may also help in monitoring the treatment [6]. However, again it is important to stress that antibiotics should be administrated only in confirmed infections and with microbiology advice. Thus, not all pregnant mares with increased blood SAA concentration need to be treated with antimicrobials, but rather high SAA concentration warrants further investigation of placentitis.

SAA in adult internal medicine

SAA concentrations have been shown as useful for screening and preliminary diagnosis in several internal disorders. Very high, reaching 1000 mg/L, SAA concentrations have been documented in horses with *Streptococcus zooepidemicus* infections. SAA concentrations and rectal temperature increased on the first day after inoculation into the lung, peaked on the third day post-infection and then decreased gradually, in most cases returning to normal value in parallel disappearance of clinical signs. The rate and amplitude of the increase was higher for SAA than fibrinogen concentration [16].

Viral infections have been shown to produce a less marked SAA response and the pattern may vary among individuals. In experimental equine coronavirus (ECoV) infection SAA has been shown to reflect the clinical status of horses. High, exceeding 100 mg/L SAA concentrations were observed from days 2 to 7 post-infection in two horses which developed biphasic fever, anorexia and pasty faeces. In contrast, no changes in SAA concentration occurred in a third horse that did not develop any clinical signs in response to the same experimental infection [45]. Similarly, in horses naturally infected with equine influenza virus (EIV), SAA concentrations increased above 100 mg/L during the first 48 h after clinical signs appeared. SAA concentrations related to clinical severity and returned to baseline within 11–22 days in uncomplicated cases [8]. In pony mares experimentally infected with EHV-1, the pattern of changes in SAA concentration was similar, it exceeded 100 mg/L 48 h postchallenge, paralleling increases in rectal temperature, and returned to normal concentrations about 10 days post-infection. In the EHV-1 model, a second rise about 10 mg/L occurred at parturition but was not accompanied by clinical signs [5]. But in one mare, which aborted, SAA concentration increased at that time to about 500 mg/L [11]. In contrast, in Standardbred trotters with subclinical respiratory disorders monitored by qPRC for equine influenza, equine arteritis virus, equine rhinitis B virus (ERBV), EHV-1 and EHV-4 and serology for equine rhinitis A virus (ERAV), ERBV, EHV-1 and EHV-4, no associations were found between increased SAA concentrations and viral activity. Only one exception, a horse with high SAA concentration and high titres to ERBV, EHV-1 and EHV-4 was noted [33].

SAA measurements in blood are considered mainly to assist in the differentiation between bacterial respiratory diseases, indicated by very high SAA concentrations and inflammation relating to other pathogens, including viral and noninfectious conditions, e.g. allergic reactions. In racehorses with mild form of equine asthma (previously described as inflammatory airway disease—IAD), recognised as a multifactorial condition, involving both viral and environmental components, SAA concentrations were similar to the values measured in healthy individuals [25]. In another study, horses with mild equine asthma had only slight, 3.5-fold, increases in blood SAA concentrations compared with healthy controls [46]. On the other hand, SAA was transiently, but significantly (at least 5 times baseline) increased by antigen challenge in heaves-affected horses [47]. Together these studies suggest that SAA concentration helps in the distinction between bacterial pneumonia and other respiratory tract conditions with similar clinical signs. However, SAA concentrations are not diagnostic in isolation and simply warrant further diagnostic testing. Particular care must be taken when deciding to use antimicrobial agents and such decisions should not be solely based on SAA concentrations but should be combined with other diagnostic testing. An accurate diagnosis is critical for the responsible use of antimicrobials.

Gastrointestinal diseases associated with inflammatory processes, such as enterocolitis, colitis, abscessation and peritonitis, have also been shown to produce increases in SAA concentrations which are absent in horses with strangulating and nonstrangulating intestinal obstructions [18,48]. Interestingly, SAA concentrations were increased (median 50 mg/L) in equine grass sickness, a neurodegenerative disease [19]. A large study involving 718 horses suggested that SAA concentration measured at admission may be a useful prognostic indicator and horses with inflammatory diseases (enteritis, colitis or peritonitis) with SAA concentrations exceeding 50 mg/L had poorer prognosis for survival than horses with other conditions [18]. However, in another study, SAA concentration at admission exceeded 20 mg/L in both survivors and nonsurvivors among colitis and peritonitis patients, but increased SAA concentration occurred between 24 and 72 h of hospitalisation in the horses which were subjected to euthanasia or developed complications [49]. In horses with gastrointestinal diseases increased SAA concentrations may confirm the inflammatory character of the disease. However, serial analysis is likely to be more effective as a diagnostic and prognostic tool than measurement only at admission.

In localised inflammatory processes, blood SAA concentrations are of marginal importance. Horses with ocular conditions such as uveitis and

keratitis showed no increase in plasma SAA [50]. This is not surprising, as the eye is isolated by blood-ocular barrier.

Joint diseases

Joint infection can be devastating to athletic careers for affected animals if treatment is delayed or inappropriate. Septic arthritis in adult horses occurs most commonly as a result of traumatic wounds and can become life threatening due to difficulties in clearing established infections and development of degenerative changes associated with ongoing inflammation. Diagnosis is based on clinical signs and synovial fluid analysis (total protein and total nucleated cell count), however, these methods may not allow for clear differentiation between acute nonseptic inflammation and infection. Early diagnosis is critical for rapid elimination of infection and inflammation to avoid ongoing cartilage degradation and osteoarthritis [51–53]. SAA can be measured in synovial fluid [4]. Its concentrations are not affected by repeated arthrocenteses [15,51], intra-articular administration of amikacin [51] arthroscopic lavage [52] or through-and-through joint lavage in healthy joints [52]. In healthy horses, SAA concentrations in both blood and synovial fluid are generally less than 1 mg/L [51]. A rise in SAA concentration has been proposed as useful to differentiate between septic or nonseptic intrasynovial pathology, with a cut-off value of 60.7 mg/L for septic condition [53]. However, large-scale clinical trials are lacking and in a small study involving five horses with experimentally induced septic arthritis, SAA in blood increased earlier (24 vs. 36 h) and was higher than in synovial fluid (111.9 ± 116.3 vs. 27.3 ± 40.1 mg/L) [10]. One of the study horses was given anti-inflammatory treatment (phenylbutazone) at the time of model induction which may have influenced or delayed the SAA response in this horse. Furthermore, blood SAA concentrations must be interpreted with caution in horses with concurrent inflammatory conditions, including large wounds or extensive soft tissue trauma which themselves may produce increased SAA concentrations [53]. Continuous decline in SAA concentration in sequential measurements indicates a favourable response to treatment in horses with injuries penetrating synovial structures [54].

Recent studies have also suggested that SAA has a key role in pathogenesis of the joint inflammation in horses. SAA is a potent inducer of production of matrix metalloproteinases in articular chondrocytes, which are involved in degradation of cartilage extracellular matrix. SAA has been shown to decrease the expression of cartilage-derived retinoic acid-sensitive protein which is involved in maintaining articular cartilage integrity [54]. Thus, it seems that SAA may contribute to alterations in chondrocyte metabolism with potential relevance for the joint pathologies. In race horses, injuries of bone and tendon produced no marked increase in SAA concentration, whereas injuries of the soft tissues within the musculoskeletal system resulted in increases [55].

SAA in surgical patients

Surgery triggers an APR which is reflected by changes in SAA concentration in blood [5,8]. The normal post-operative SAA response pattern involves a rise and fall [1]. A sustained postsurgical increase in SAA concentrations may indicate post-operative infection [49]. After minor surgical procedures under general anaesthesia, without post-operative infection, SAA concentrations from 100 to 400 mg/L with a peak at approximately day 3 after surgery can be expected. Procedures which have been studied include tibiotarsal arthroscopy and osteochodrosis fragment removal, laryngoplasty and ventriculectomy (peaked 50–150 mg/L at day 2; return to normal concentration by day 7), carotid exteriorisation and flexor tendon division (peaked 100–400 mg/L at day 2; return to normal concentration by 7–14 days) [11,20,56] and a variety of elective procedures including minor airway and orthopaedic surgeries (peaked 16.4 mg/L at 24 h) [57].

SAA measurement may be useful in the management of acute abdominal pain. Pihl *et al.* [58] evaluated clinical and clinical plus blood test models for differentiating horses with inflammatory colic (enterocolitis and peritonitis) from those with colic requiring surgery. The clinical model included lethargy, rectal temperature >38°C, gastric reflux 5–10 L and normal rectal examination findings. All these variables were positive predictors of inflammatory colic except for gastric reflux of 5–10 L, which was a negative predictor. The ability of this model to correctly differentiate inflammatory from surgical colic was 86% as determined by area under the receiver-operating characteristic curve. The clinical plus blood test model included also white blood cell counts, haematocrit, total plasma protein, lactate, SAA, haptoglobin and fibrinogen concentrations. With SAA included in the model no additional blood parameters improved the model, and the final area under the curve was 90%. Thus, SAA measurement improved the predictive model accuracy [58]. Serial measurement of SAA at 48, 72 and 96 h after exploratory celiotomy has also been shown to help to determine risk of complications and guide post-operative management [59]. SAA concentration indicated the onset of an inflammatory response more sensitively than fibrinogen concentration. However, some strangulating conditions may progress very quickly and it may be more useful to measure SAA in peritoneal fluid, where it increases more rapidly in horses with strangulations than in horses with colic for other reasons [60].

In experimental equine wounds healing by secondary intention, there were considerable individual variations in the concentration of SAA in serum and interstitial fluids [61]. The role of SAA measurement in clinical management of horses with wounds has not yet been documented but sequential measurements may have some potential where infection is involved.

Conclusion

SAA measurements aid in diagnosis, prognosis and general assessment of health in horses. Because concentrations are low in healthy horses and rise quickly in response to inflammation, SAA is sensitive, reliable and early indicator of inflammation. SAA measurements may be helpful in the differentiation between bacterial and viral diseases, the former typically giving rise to marked increases in plasma SAA concentrations while latter is associated with mild or moderate increases. The accessibly of SAA is useful when early diagnosis and immediate and aggressive treatment is critical such as in neonatal sepsis. However, SAA responses are nonspecific and measuring SAA concentration in isolation is not a gold standard. SAA should be used in combination with other diagnostic tests and procedures and should not be used as the sole basis for prompting antimicrobial treatment. Serial measurement of SAA concentrations can be used to assess the effectiveness of responsibly chosen antimicrobial and other treatments and document improvement.

Authors' declaration of interests

The authors declare no competing interests.

Ethical animal research

Not applicable.

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Authorship

O. Witkowska-Piłaszewicz and A. Cywińska prepared initial drafts of this review article. All authors contributed to and approved the final version.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supplementary Item 1: Serum amyloid A (SAA) concentrations reported in healthy horses.