Concentrations of serum amyloid A in serum and synovial fluid from healthy horses and horses with joint disease

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Objective—To determine serum amyloid A (SAA) concentrations in serum and synovial fluid from healthy horses and horses with joint disease and assess the effect of repeated arthrocentesis on SAA concentrations in synovial fluid.

Animals—10 healthy horses and 21 horses with various types of joint disease.

Procedures—Serum and synovial fluid samples were obtained from each horse. In 5 of the 10 healthy horses, arthrocentesis was repeated 9 times. Concentrations of SAA were determined via immunoturbidimetry.

Results—Serum and synovial fluid SAA concentrations were less than the assay detection limit in healthy horses and did not change in response to repeated arthrocentesis. Synovial fluid SAA concentrations were significantly higher in horses with suspected bacterial joint contamination or infectious arthritis, or tenovaginitis than in healthy controls, and serum concentrations were significantly higher in horses with infectious conditions than in the other groups. Neither serum nor synovial fluid SAA concentrations in horses with low-inflammation joint conditions differed significantly from those in healthy controls. Concentrations of SAA and total protein in synovial fluid were significantly correlated.

Conclusions and Clinical Relevance—Synovial fluid SAA concentration was a good marker of infectious arthritis and tenovaginitis and appeared to reflect changes in inflammatory activity. The advantages of use of SAA as a marker include the ease and speed of measurement and the fact that concentrations in synovial fluid were not influenced by repeated arthrocentesis in healthy horses. Further study of the SAA response in osteoarthritic joints to assess its usefulness in diagnosis and monitoring of osteoarthritis is warranted. (Am J Vet Res 2006;67:1738–1742)

Serum amyloid A is an acute-phase protein that is synthesized primarily in the liver in response to infection and inflammation. The protein has been highly conserved throughout evolution and is thought to play important roles in modulation of the host response during infections and inflammatory disease conditions in several vertebrate species.1 It is a sensitive acute-phase reactant in horses and humans, and concentrations may increase several hundred times in response to infection and tissue injury.2–4 Inflammatory cytokines such as interleukin-1β, interleukin-6, and tumor necrosis factor-α are responsible for upregulation of SAA synthesis.1

Studies of the SAA response to joint disease in horses are limited, but it is known that SAA concentrations increase in serum as well as in synovial fluid after experimental induction of arthritis.6,7 It has been suggested that SAA is synthesized locally in the inflamed joint in horses,7 similar to findings reported8,9 in humans and rabbits. In humans, the SAA response in naturally occurring joint disease has mostly been investigated in association with rheumatoid arthritis.10,11 The SAA response in humans with osteoarthritis has been described in a limited number of studies,12,13 but to the authors’ knowledge, the SAA response to infectious arthritis in horses has never been described.

It has been suggested that in humans, serum SAA concentration is a more sensitive marker of joint inflammation than erythrocyte sedimentation rate and concentration of the other major acute-phase protein, C-reactive protein14,15; therefore, measurement of serum SAA concentration may be useful for monitoring changes in disease activity in humans with rheumatoid arthritis.11,12

We hypothesized that serum and synovial fluid concentrations of SAA, the major acute-phase protein in horses, would be increased in horses with naturally occurring joint disease and that horses with joint disease characterized by a strong inflammatory component (eg, septic arthritis) would have higher SAA concentrations than horses with less inflammatory joint diseases (eg, osteochondrosis and chronic osteoarthritis). The study objectives were to measure concentrations of SAA in serum and synovial fluid from healthy horses and horses with naturally occurring joint disease and assess the effect of repeated arthrocentesis on synovial fluid SAA concentrations, with the overall purpose of evaluating the clinical usefulness of this marker in horses with joint disease.

Materials and Methods

Animals and samples—Eighty-nine synovial fluid samples (from joints or tendon sheaths) and 73 serum samples stored in 1-mL aliquots at –20°C at The Royal Veterinary and Agricultural University were available for analysis. The samples were derived from 31 horses. Samples from 5 control
horses (group 1A) were obtained during a study\(^1\) for which the experimental protocol was approved by the Danish Animal Experimentation Inspectorate and in which all procedures were conducted according to the Danish Animal Testing Act. All other samples were obtained at the discretion of the attending clinician as part of routine evaluation (n = 5) or lameness evaluation or treatment (n = 21) from client-owned horses admitted to the university clinic from May 2003 to August 2005. All horses were admitted to the university clinic under general informed consent that samples obtained during routine clinical procedures may be used for research purposes.

Ten horses with no evidence of joint disease (as determined by lameness examination, flexion tests, ultrasonographic imaging, and synovial fluid analysis) were designated as healthy controls (group 1). Samples were obtained from 5 of the control horses (group 1A) during a study on experimentally induced arthritis (the horses served as healthy controls in that study also). Synovial fluid samples were obtained from the radiocarpal joints of those horses before and 4, 8, 12, and 24 hours after intra-articular injection of 2 mL of isotonic saline (0.9% NaCl) solution into the joint. The other 5 controls were client-owned horses that were euthanatized for nonorthopedic reasons (group 1B). Group 2 (n = 5) was composed of horses in which bacterial joint contamination was possible because of a wound that penetrated to a joint or a fracture that communicated with or occurred in close proximity to a joint. This group was heterogenous, but all horses in group 2 had synovial fluid leukocyte counts less than the reference limit (< 0.5 × 10\(^9\)/L). Group 3 (n = 7) was composed of 6 horses with infectious arthritis and 1 horse with infectious tenovaginitis. Those diagnoses were made on the basis of clinical findings (eg, severe lameness and joint distension), changes in synovial fluid (eg, cloudy appearance, watery consistency, or both), or a high synovial fluid leukocyte count and total protein concentration. Results of bacterial culture were negative in all horses, which was considered to be a result of the difficulty in culturing microorganisms from synovial fluid.\(^1\)\(^2\) Samples were plated on blood agar, and it is possible that use of blood culture vials would have yielded bacterial growth. Group 4 (n = 9) was composed of horses with conditions that affect the joint but that are not typically accompanied by a systemic inflammatory response. Seven of those horses had osteoarthritis. Osteoarthritis was diagnosed on the basis of clinical examination; results of radiographic or ultrasonographic examination (with findings of osteophyte formation, capsular fibrosis, and cartilage thinning); and, in 5 horses, postmortem findings. The other 2 horses in group 4 had osteochondrosis; diagnosis in those horses was made on the basis of radiographic examination and was confirmed by arthroscopy.

Blood was collected by venipuncture of a jugular vein, and serum was separated by centrifugation (2,500 × g for 15 minutes at ambient temperature). After blood samples had been obtained, horses were sedated for the arthrocentesis procedure with detomidine (0.01 mg/kg, IV) and butorphanol tartrate (0.02 mg/kg, IV). Synovial fluid was obtained by means of arthrocentesis. An aliquot of synovial fluid was submitted for bacterial culture, and the remainder of the sample was immediately transferred to tubes containing EDTA. One aliquot of the EDTA-stabilized synovial fluid sample was submitted for leukocyte count, and the remainder of the sample was centrifuged (2,500 × g for 15 minutes at ambient temperature) and the supernatant immediately frozen. Serum and synovial fluid samples were stored at −20°C until SAA and total protein determination.

**Laboratory analyses**—White blood cell counts were determined in EDTA-stabilized blood by use of an automatic cell counter and in EDTA-stabilized synovial fluid by counting cell numbers in a hemocytometer with a microscope. Total protein concentration in EDTA-stabilized synovial fluid samples was determined by use of refractometry.\(^1\)\(^3\)

Bacterial culture was performed by inoculating synovial fluid samples on 5% bovine blood agar and incubating for 1 to 2 days at 37°C. Serum and synovial fluid concentrations of SAA were measured with a commercially available immunoturbidimetric assay by means of a described method.\(^1\)\(^4\) Isoforms of SAA in serum and synovial fluid were detected by use of denaturing isoelectric focusing and western blot analysis according to a described method.\(^1\)\(^5\) Briefly, serum and synovial fluid samples were diluted in 8M urea and separated by isoelectric focusing on dried gels\(^1\)\(^6\) reconstituted with a mixture of 8M urea and preblended ampholine (pH, 3.5 to 9.5) according to the manufacturer's instructions (separation technique file No. 101). After separation, semi-dry western blotting onto a nitrocellulose membrane was performed, and SAA was stained with a biotinylated monoclonal anti-SAA antibody.\(^1\) To determine the apparent isoelectric point of the SAA isoforms, serum and synovial fluid samples were separated on a gel along with proteins with known pl value.\(^1\)

**Statistical analysis**—Nonparametric Kruskal-Wallis analysis was used to analyze overall differences in serum and synovial fluid SAA concentrations and synovial fluid total protein concentration among groups. Pairs of group medians were compared by use of the Dunn multiple comparison test. Repeated-measures ANOVA was used to analyze changes in synovial fluid total protein concentration at different time points in control horses that underwent repeated sampling (group 1A), and the Bonferroni multiple comparison test was used to compare concentrations in synovial fluid after the first arthrocentesis with those in synovial fluid samples obtained after subsequent arthrocentesis procedures. The correlation between SAA and total protein concentrations in synovial fluid was tested by calculating the Spearman correlation coefficient. For all comparisons, P < 0.05 was considered significant.

**Results**

**SAA concentrations in serum and synovial fluid**—In all serum samples from healthy controls (groups 1A and 1B), SAA concentrations were less than the assay detection limit (0.48 mg/L; Figure 1). In 51 of 53 synovial fluid samples from group 1, concentrations of SAA were less than the detection limit, and in the remaining 2 samples, SAA concentrations were 0.6 and 0.7 mg/L (Figure 2). Concentrations of SAA in synovial fluid did not change in response to repeated arthrocentesis in horses in group 1A, whereas total protein concentration increased significantly (P < 0.001) in synovial fluid obtained in the second arthrocentesis procedure (at 4 hours) and remained significantly increased until the last arthrocentesis procedure at 144 hours (Figure 3).

Total protein concentrations in synovial fluid differed significantly (P < 0.001) among groups, with concentrations in group 2 (median, 3.8 g/dL) and group 3 (median, 4.0 g/dL), but not in group 4 (median, 1.3 g/dL), being significantly higher than those in group 1 (median, 0.55 g/dL). Serum and synovial fluid SAA concentrations differed significantly (P = 0.015 and P = 0.0015, respectively) among groups. Concentrations of SAA in serum were significantly (P < 0.05) higher in group 3 than in group 1 (Figure 1), and concentrations in synovial fluid were significantly (P < 0.05 and P < 0.001, respectively) higher in group 2 and 3 than in...
Neither serum nor synovial fluid SAA concentrations in group 4 differed from those in group 1. Concentrations of SAA and total protein in synovial fluid samples were significantly correlated (P < 0.001; Spearman correlation coefficient, 0.57).

Concentrations of SAA in serum and synovial fluid ranged from below detection limit to 402 and 94.5 mg/L, respectively, among horses in group 2 (Figures 1 and 2). The highest concentrations were detected in a horse with an open infected fracture of the second metacarpal bone in close proximity to the radiocarpal and intercarpal joints. Two horses in group 2 had serum and synovial fluid SAA concentrations less than the detection limit: in 1 of those horses, arthrocentesis had been performed 2 hours after injury, and the other horse had a closed fracture involving the radiocarpal joint. Two horses (1 with a wound that penetrated to the radiocarpal joint and 1 with a wound that penetrated to the talocrural joint) had low initial SAA concentrations in synovial fluid that decreased further during the course of treatment with joint lavage and administration of antimicrobials and nonsteroidal anti-inflammatory drugs (Figure 4).

Four horses with active infectious arthritis or tenovaginitis had moderate to high SAA concentrations in serum and synovial fluid (Figures 1 and 2). However,
Serum amyloid A isoforms in serum and synovial fluid—Isoelectric focusing revealed 3 major and as many as 4 minor SAA isoforms in serum (Figure 5). The apparent pl values of the 3 major serum isoforms interpolated from the standard curve were 7.9, 8.6, and > 9.3 (9.6 by extrapolation from the standard curve). Two additional isoforms with highly alkaline apparent pl values (10.0 and 10.2 by extrapolation from the standard curve) were detected in synovial fluid samples. These isoforms were not detected in any of the serum samples.

Discussion

Serum amyloid A was not detected in serum or synovial fluid of healthy horses, even after repeated arthrocentesis. In contrast to SAA, the synovial fluid total protein concentration increased within 4 hours of arthrocentesis and remained high in response to arthrocentesis performed every 24 to 48 hours. These characteristics may constitute an advantage in determining synovial fluid SAA concentrations over total protein concentration or other, newer markers of joint inflammation such as nitric oxide, prostaglandin, and collagenase 1; the latter may be detected in synovial fluid from healthy joints, and concentrations of those markers in joint fluid are influenced by previous arthrocentesis.6,7 A further advantage of use of SAA is the ease and speed with which it can be measured (results are available in < 30 minutes).18,22

Horses suspected of having bacterial contamination of the joint (group 2) had various SAA responses, a finding that may reflect the heterogeneity of the group. One horse in that group in which no SAA was detected in serum or synovial fluid had undergone arthrocentesis and synovial fluid sampling for analysis 2 hours after injury. It is possible that this was not sufficient time for SAA to increase to a detectable concentration in the synovial fluid because it is known that SAA concentrations in synovial fluid do not increase until 4 to 8 hours after experimental induction of arthritis.7 In contrast to horses in group 2, horses with active infectious arthritis or tenovaginitis (group 3) had high SAA concentrations in serum and synovial fluid. An increase in synovial fluid SAA concentration has been reported in horses and humans with experimentally induced or naturally occurring aseptic inflammatory arthritis,5,10 but to the authors’ knowledge, the present study is the first in which the SAA response in horses with infectious arthritis or infectious tenovaginitis has been described. Synovial fluid samples from 3 horses in group 3 contained no or only very low concentrations of SAA. One horse had received an intra-articular corticosteroid injection 12 days before sampling of synovial fluid, and this anti-inflammatory treatment may have affected the SAA response. Synovial fluid from the other 2 horses was obtained only after treatment of the joint condition, at which point inflammation may have subsided (as suggested by synovial fluid leukocyte counts within reference range [data not shown]), resulting in low SAA concentrations.

The detection of joint-specific SAA isoforms in horses with infectious arthritis corroborated results from a study of horses with experimentally induced inflammatory arthritis.7 The SAA measured in synovial fluid may be derived from 2 sources: hepatically synthesized protein that gains access to synovial fluid from the blood and intra-articularly synthesized protein that is released directly into synovial fluid. Results of isoelectric focusing suggested that SAA in synovial fluid is derived from a combination of those 2 sources. Synovial fluid contained the 3 major isoforms of the protein that are also found in serum (ie, hepatically synthesized SAA) as well as 2 joint-specific isoforms (likely synthesized by chondrocytes or synoviocytes, as has been reported in humans and rabbits8,9).

In group 4 horses, which had joint disease but no systemic inflammatory response, SAA concentrations in serum and synovial fluid were low or absent. The fact that more synovial fluid than serum samples had
SAA concentrations above the detection limit may reflect that mild inflammation (ie, sufficient for stimulation of a local SAA response) was generated intra-articularly but that osteoarthritic processes do not cause systemic inflammation and hence do not induce hepatic SAA synthesis. All of the horses with osteoarthritis had cartilage or bone changes consistent with chronic osteoarthritis. Serum amyloid A is synthesized only in response to active inflammation, which could explain the low number of synovial fluid samples that contained SAA in group 4 horses.

In humans with osteoarthritis, only slight increases in serum and synovial fluid SAA concentrations relative to healthy controls have been reported, but it has nevertheless been suggested that SAA potentially is a more sensitive and earlier marker of joint damage than radiographic imaging and is a better indicator of disease activity and prognosis than assay of cartilage breakdown products. It has also been suggested that even small changes in SAA concentration may represent clinically important changes from the healthy state and warrant further diagnostic testing and treatment; further investigation of the usefulness of SAA in diagnosis and management of osteoarthritis in horses is therefore warranted.

Concentrations of SAA in serum and synovial fluid may reflect intra-articular inflammatory activity in horses with arthritis, and Cunnane et al found that SAA concentrations decrease during stages of clinical improvement and increase with clinical deterioration of joint disease in humans. Results of the present study suggest that measurement of SAA concentration in synovial fluid may be useful for monitoring changes in intra-articular inflammatory activity that results from treatment of joint contamination or infection because SAA concentrations decreased during the course of treatment of these conditions in some horses.

It was not possible to assess the usefulness of SAA determination in detection and monitoring of osteoarthritis on the basis of data from the present study. Future studies should include analysis of synovial fluid from horses with infectious arthritis in the acute and subacute stages, and studies in which the positive and negative predictive values of serum and synovial fluid SAA measurements are determined are necessary before recommendations on the use of this marker as an aid to diagnosis in horses can be made.

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References