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ORIGINAL RESEARCH

Using systemic serum amyloid A as a biomarker for synovial structure infections in horses with acute limb wounds

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Abstract

Background: In postoperative monitoring of synovial structure infection due to limb wounds, early recognition of a recurrence of synovial infection is indispensable to prevent further damage to the affected synovial structure. This study evaluated the role of serum amyloid A (SAA) as a systemic biomarker in disease monitoring and correlated this tool with clinical variables.

Methods: In this prospective cohort study, 55 horses with acute limb wounds were divided into two groups: those with (group 1, $n = 26$) or without (group 2, $n = 29$) a diagnosis of synovial structure penetration. SAA, lameness and body temperature were evaluated repeatedly and compared between groups. Correlations were explored between SAA and body temperature as well as lameness. The long-term outcome was also analysed.

Results: In both groups, SAA levels followed the characteristic rise-and-fall pattern observed in previous studies, with a significant increase up to a peak concentration within 48 hours, followed by a constant decline. Lameness and body temperature did not change significantly. SAA was not found to correlate with clinical variables at all time points. Three horses in group 1 had a recurrence of synovial sepsis with an associated increase in SAA. The long-term outcome was good. A total of 71% of the study population returned to pre-injury performance levels.

Conclusion: Repeated measurements of SAA accurately reflected the course of synovial inflammation and thus provided a reliable and rapidly available tool to monitor the disease course and to adapt the treatment regimen. SAA should be routinely added to the postoperative management of such cases.

KEYWORDS
disease monitoring, equine, limb injuries, serum amyloid A, synovial structure infection, wound

INTRODUCTION

Synovial structure infection is a common complication of acute wounds in the limb region and can lead to chronic lameness, limitation of the intended performance level or complete retirement from athletic activities.[1–4] During postoperative monitoring, early recognition of recurrence is indispensable to prevent further damage of the affected synovial structure, such as cartilage destruction, osteoarthritis or intrathecal adhesions, and to significantly improve the outcome.[1–6] In response to synovial infection, matrix-metalloproteinase activity is stimulated in order to destroy invading foreign material and demarcate the surrounding damaged tissues.[2,3,7] The synovial hyaluronic acid concentration decreases, and glycosaminoglycans and collagen are lost from the extracellular matrix. Concurrently, pathogens produce toxins and proteolytic enzymes, which trigger the inflammatory reaction.[2,3,7,8] As an inflammatory product, fibrin is released in large amounts.[9] Immediate treatment with arthroscopic lavage of the affected synovial structure and initiation of antimicrobial and anti-inflammatory medication are advised to limit these processes.[1,2,4,5,10,11]

Routine monitoring of the disease course with therapy adaptation consists of evaluating the inflammatory situation within the synovial structure by

assessing clinical signs such as lameness, fever, local pain and swelling of the affected area; alternatively, it is adjusted based on the results of repeated synovial fluid (SF) analyses.[1,5,7–9,11–15] The duration of postoperative systemic treatment with antimicrobials as well as anti-inflammatory and analgesic drugs is commonly based on the clinical signs, synoviocentesis or the personal experience of the treating clinician.[1,4,5,7–9,11,16] However, clinical signs are not always manifest or clear.[5,17]

Repeated synoviocentesis enables the evaluation of synovial parameters such as total nucleated cell count (TNCC), percentage of neutrophils (%*N*) and total protein (TP).[5,7,11–13,15] However, repeated synovial puncture can be problematic as a strategy because of the need for repeated bandage or cast changes, difficulties sometimes collecting SF and the potential risk of iatrogenic infection when there is diffuse subcutaneous swelling.[18] In addition, repeated synoviocentesis, arthroscopic lavage or application of intrasynovial medication has an impact on synovial TP, TNCC and %*N*, and thus these values may not give a clear answer regarding the resolution of the infection; however, systemic serum amyloid A (SAA) is not affected.[13,19–21]

An evaluation of the systemic inflammatory reaction can be used to assess the local inflammatory situation. As a major acute phase protein in the horse, SAA has already proven to be a helpful marker in postoperative monitoring after castration and minimally invasive procedures[22–24] as well as in synovial sepsis.[13–15,25] It can be used as an index of the patient's recovery from various infectious or inflammatory conditions (bacterial or viral pneumonia, colic, neoplasia).[22–24,26] In horses, SAA is well suited for the real-time monitoring of disease activity because SAA levels accurately reflect the severity of inflammation.[17,23,24,26] The effect of treatment can also be observed by evaluating the course of SAA.[14] The half-life of SAA is short, with reported values of approximately 90 minutes[27,28]; thus, concentrations decrease as soon as the therapy is successful. Postoperative SAA levels are independent of preoperative values, whereas levels of white blood cells (WBCs) and fibrinogen depend on preoperative concentrations.[26] In septic synovitis, physiologically low SAA plasma concentrations show a typical pattern, which starts to increase after 16 hours, peaking 2–3 days after insult with concentrations of up to 1000-fold from baseline and returning to preoperative levels, following clinical progression, within an average of 7–10 days under successful therapy.[13,29–32] Deviations from this pattern may indicate postoperative complications such as recurrence of infection, lack of treatment response, surgical site infections or secondary comorbidities.[14,22,26,29]

The aim of this study was to analyse the role of SAA as an objective, systemic biomarker for disease progression and treatment response in horses with synovial structure infection due to acute wounds in the limb region. The course of SAA was compared between horses with and without a diagnosis of synovial structure infection. Correlations between SAA levels and clinical variables were also analysed. Furthermore, the long-term outcome was evaluated. In addition, the utility of adjusting the duration of antimicrobial therapy to SAA levels was evaluated.

MATERIALS AND METHODS

Horses

The ethics committee of the regional council of Giessen, Hessen, Germany did not consider this study as an animal experiment (reference number kTV 6-2018). As such, no ethical approval was required. All blood samples were collected during routine diagnostic work-up of the cases. Owners gave their informed consent to use the results for the research purposes of this study.

Fifty-five horses of mixed breed, age and sex (Table 1) that had been presented to the equine clinic (surgery, orthopaedics), Justus-Liebig-University of Giessen, for the treatment of acute wounds in the limb region, which had occurred less than 24 hours before admission, were included in this prospective cohort study. Injuries included wounds with suspected synovial structure involvement. Horses were included provided there were no other abnormalities on clinical examination at admission that indicated an acute infection. Horses with other signs of acute infection or inflammation were excluded. Horses were also excluded if they developed any disease unrelated to the trauma during the study period.

For the analysis, horses were divided into two groups. Group 1 (G1) represented the subject group and consisted of horses with a confirmed diagnosis of synovial structure penetration due to the injury. Group 2 (G2) represented the reference group and included horses with no involvement of any synovial structure related to the injury. Diagnosis or exclusion of synovial structure involvement was based on the results of an initial physical and blood examination (SAA, WBC, fibrinogen), SF analysis (gross appearance with viscosity, colour and turbidity; TNCC > 30×10^9 cells/L; %*N* > 90%; TP > 25 g/L), synovial pressure-leak testing, radiographs and/or ultrasonography. For the diagnosis of synovial infection, at least two of the synovial parameters had to be abnormal, or synovial pressure-leak testing or radiographs indicated a communication of the injury with the synovial cavity or ultrasound revealed signs of synovial infection. The results were interpreted in context. Horses were assigned to G2 if none of the abovementioned criteria for synovial sepsis were applied. Bacterial culture and susceptibility testing of synovial samples was performed to guide antimicrobial therapy. A history of pretreatment with antimicrobials and non-steroidal anti-inflammatory drugs (NSAIDs) was recorded.

TABLE 1 Summary of the demographic information of the horses included in the study

Group	Number of horses	Median age in years (range)	Sex (*n*)	Breed (*n*)
1 (diagnosis of synovial structure involvement)	26	9 (0.2–22)	Gelding (11) Mare (11) Stallion (4)	German warmblood (11), pony (4), Arabian horse (4), Quarter horse (2), Paint (1), Haflinger horse (1), Andalusian horse (1), Fjord horse (1), mix (1)
2 (without synovial structure involvement)	29	10 (0.3–24)	Gelding (12) Mare (16) Stallion (1)	German warmblood (19), pony (2), Arabian horse (2), Quarter horse (2), Thoroughbred (1), Haflinger horse (1), Andalusian horse (1), mix (1)

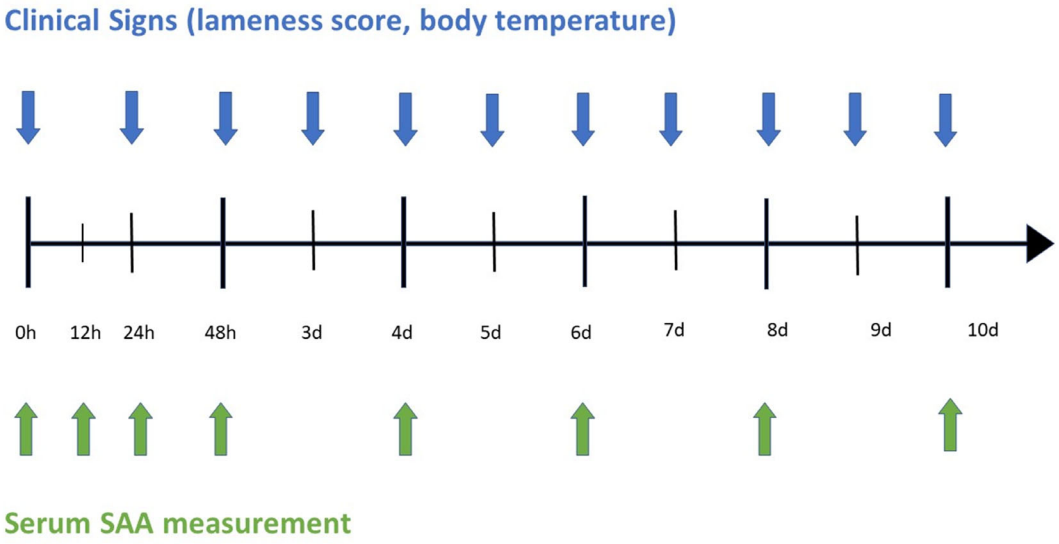

FIGURE 1 Pattern of horse monitoring with time points of routine serum amyloid A (SAA) determination and assessment of clinical variables

Monitoring

Systemic serum amyloid A

Serum samples were collected for SAA determination at admission (before surgical treatment), 12, 24 and 48 hours afterwards, and subsequently every 48 hours until SAA reached baseline (Figure 1). Serum was obtained by collecting blood in tubes with a clotting activator (Serum tube, Sarstedt, Nuembrecht, Germany) and by letting blood samples coagulate for 1 hour following centrifugation at 3000 x *g* for 10 minutes. Afterwards, samples were used immediately for analysis or stored at −20°C for a maximum time of 63 hours before analysis. Serum SAA concentrations were determined by the immunoturbidometric method (LZ test SAA, Eiken Chemical, Tokyo, Japan). A cut-off value of 2.7 µg/ml was used for the baseline, based on the reference range determined by the Department of Veterinary Clinical Sciences, Clinical Pathology and Clinical Pathophysiology, Justus-Liebig-University Giessen.

Clinical criteria

Daily monitoring of each patient included an assessment of lameness and measurement of rectal temperature (°C; Figure 1). Lameness was assessed at walk and scored as sound (grade 0) or with mild (score 1), moderate (score 2) or profound (score 3) lameness, depending on the extent of weight bearing of the affected limb at each step (according to the American Association of Equine Practitioners [AAEP] lameness scale; score 1 = AAEP grade 3, score 2 = AAEP grade 4, score 3 = AAEP grade 5). Assessments were carried out by a trained veterinarian specialising in orthopaedics. Body temperature was taken by rectal measurement.

Long-term outcome

The long-term outcome was determined for each patient by telephone interview with the owner approximately 2 years after discharge. A successful long-term outcome was defined as a horse that, in the owner's

TABLE 2 Staging of long-term outcomes (designed in accordance with Taylor et al.[33])

Stage	Description
1	Horse returned to preinjury performance or higher level of function
2	Horse was sound, but not used at previous performance/used at poorer level
3	Horse still had problems with aseptic synovitis after treatment, but lameness resolved without further treatment
4	Horse was subjected to retirement or euthanasia due to conditions related to the involved synovial structure or the soft tissue trauma
5	Horse was subjected to retirement or euthanasia due to unrelated conditions
6	Horse was lost to follow-up

opinion, was performing at or above the level of performance that it had achieved prior to the soft tissue trauma (Table 2). The staging system was designed in accordance with Taylor et al.[33]

Statistical methods and data analysis

A priori sample size calculation revealed a minimum sample size per group of 23 horses. Data were tested for normality with the Shapiro–Wilk test. SAA values were non-normally distributed and thus $\log_{10}$ transformed to stabilise the statistical model. The description of a parameter within a group over time was calculated by a paired *t* test. For the group comparison of SAA and body temperature, ANOVA with repeated measurements according to time was used, including the fixed effect of group. Group comparison over time for lameness as an ordinal parameter evaluated by a scoring system was performed by first calculating the area under the curve (AUC) for each horse and then using a Wilcoxon–Mann–Whitney test to compare the median AUC values of each group. Correlations between serum SAA concentrations and body temperature were tested by calculating Pearson's correlation coefficient (r) within each group. Spearman's correlation coefficient (r_s) was determined to investigate the correlation between serum SAA concentrations and lameness. To determine the average length of therapy with antimicrobials and NSAIDs as well as SAA values needed to reach baseline values, a Kaplan–Meier survival analysis with censored data was carried out. Data were censored because the horses were discharged as soon as they were sufficiently recovered from surgery, and thus the amount of data available declined towards the end of the study period. The survival curves were compared between the two groups using a log-rank test.

A p-value less than 0.05 was considered to indicate statistical significance. Statistical analysis and graphical presentation of data were performed using statistical software SAS 9.4 (SAS Institute, 2013), Microsoft Excel (Microsoft Corporation, 2016) and GraphPad Prism 9.1.2 (GraphPad Software, 2020).

RESULTS

Horses, surgical treatment and medication

Fifty-five horses, of which 26 were allocated to G1, were included in this study (Table 1). One horse developed thrombophlebitis and was excluded from the study. Surgery typically consisted of standard wound debridement and arthroscopic lavage of the affected synovial structure under general anaesthesia (GA). However, several patients in G2 underwent wound debridement and closure under standing sedation with local anaesthesia. Patients received 1.1 mg/kg flunixin meglumine orally (PO) or intravenously (IV) (twice daily; 51 horses), 0.6 mg/kg meloxicam PO or IV (once daily; three horses) or 4.5 mg/kg as a loading dose on the first day, followed by 2.5 mg/kg phenylbutazone PO or IV (twice daily; one horse). In addition, all patients were initially treated with systemic broad-spectrum antimicrobials, which were adjusted according to a susceptibility test, if results were available: 10 mg/kg amoxicillin (twice daily, IV or intramuscularly [IM]), preferentially in combination with 6.6 mg/kg gentamicin (once daily, IV), 1 mg/kg cefquinome (once daily, IM), 2 mg/kg marbofloxacin (once daily, IV or IM) or 30 mg/kg trimethoprim-sulphadiazine (twice daily, PO). If involvement of anaerobic bacteria was suspected, systemic treatment with 25 mg/kg metronidazole (PO twice daily) was added. In the case of synovial structure involvement, the affected synovial structure was treated intrasynovially with 250–500 mg amikacin per structure subsequent to joint lavage.

Postoperative management

Following surgical intervention, medication was continued or, if results were available, antimicrobials were adapted to the susceptibility test. NSAIDs were given for at least 5 days until lameness improved and/or fever resolved. Overall, 50% of the horses in G1 received NSAIDs for 9.5 days (95% confidence interval [CI] = 6–13 days), whereas in G2, 50% were medicated with NSAIDs for 7 days (95% CI = 5–7 days). This difference was statistically significant ($p = 0.002$). The duration of the systemic treatment with antimicrobials was adjusted to SAA serum concentration as an indicator of infection; thus, antimicrobials were administered until SAA serum concentrations reached baseline values. In G1, 50% of the horses were medicated with antimicrobials for 12 days (95% CI = 11–15 days). This was significantly longer ($p < 0.0001$) than for the horses in G2, in which 50% of horses were treated with antimicrobials for 7 days (95% CI = 6–8 days). Horses were kept on strict stall rest, except for a daily short walk to assess lameness. The affected area involving

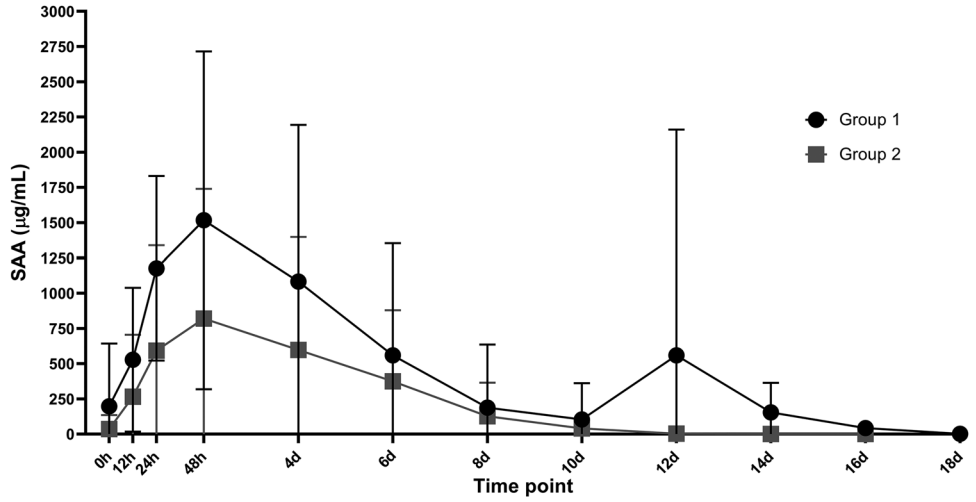

FIGURE 2 Course of serum amyloid A (SAA) concentrations (µg/ml; mean and SD) of groups 1 and 2 during the observation period

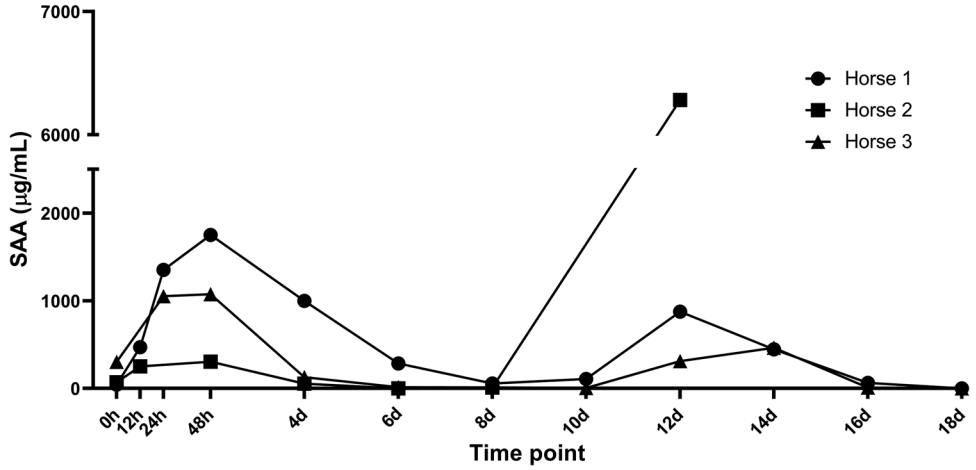

FIGURE 3 Course of serum amyloid A (SAA) concentrations (µg/ml) in three horses with recurrence of septic synovitis

the synovial structure and the wound was protected by a sterile bandage or cast for immobilisation. Bandage change was performed every 2–3 days or as needed.

SAA course

In G1, the initial mean SAA concentrations of 196.59 µg/ml showed a significant ($p < 0.0001$; related to the $\log_{10}$ values) increase within 48 hours to maximal concentrations of 1517.3 µg/ml (range 301.3–6174 µg/ml; Figure 2). Afterwards, SAA concentrations began to decline constantly. Fifty percent of the horses in G1 reached baseline values (<2.7 µg/ml) within 12 days (95% CI = 11–15 days). The second peak of the mean SAA concentration on day 12 in G1 was due to three horses that had a recurrence of synovial sepsis (Figure 3). The first horse (case number 16, Table S1), in which the digital flexor tendon sheath was involved, showed a severe inflammatory reaction with a high increase in SAA concentration up to 1751.3 µg/ml at 48 hours. On day 8, SAA had decreased to 57.8 µg/ml. On day 10, SAA levels started to increase, peaking on day 12 at 876.5 µg/ml (Figure 3). This coincided with deteriorating lameness but not fever. The second horse (case number 22, Table S1) manifested septic arthritis of the talocrural joint. The mild initial SAA peak of 305.1 µg/ml was followed by a decrease until day 8, when the horse was discharged. On day 12, the horse was readmitted because of fever and profound lameness. Severe recurrence of septic inflammation with an SAA value of 6383 µg/ml was apparent (Figure 3). In the third horse (case number 25, Table S1), which also had involvement of the digital flexor tendon sheath, a moderate initial SAA increase up to 1075.7 µg/ml was observed, after which concentrations decreased. On day 12, the SAA concentration increased to 312.2 µg/ml, and fever and moderate lameness occurred. After a second surgical therapy, the SAA decreased from day 16 onwards (Figure 3). In all three cases, the renewed increase in SAA concentrations indicated a need for further diagnostics, including synoviocentesis. Recurrence of synovial infection was thus confirmed, and a second arthroscopic lavage was initiated. In the second surgical intervention in the second case, the arthroscopic findings of the involved talocrural joint revealed a poor prognosis, so the horse was euthanized.

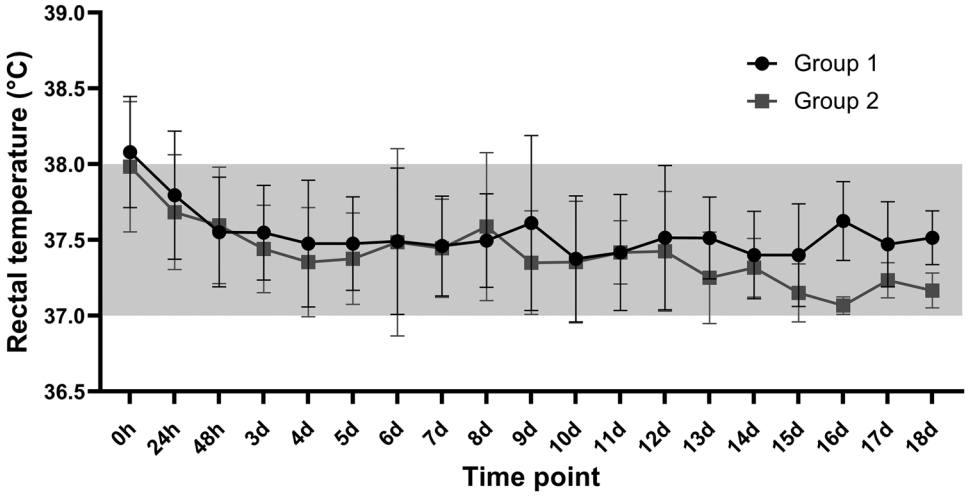

FIGURE 4 Course of body temperature (°C; mean and SD) of groups 1 and 2 during the observation period. The normal range is grey shaded (37.0°C–38.0°C)

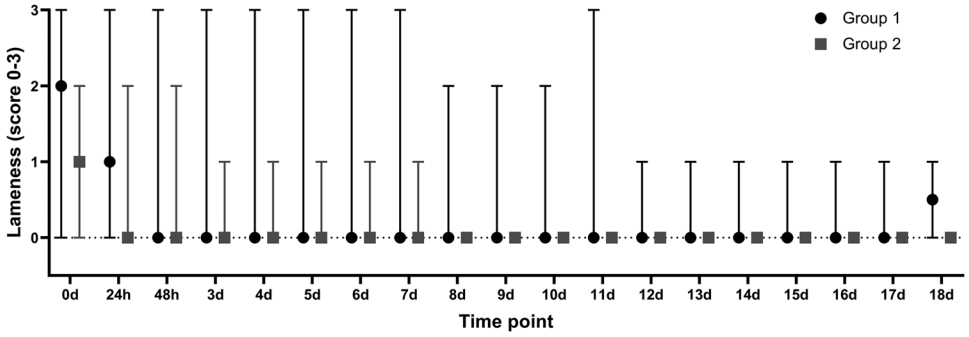

FIGURE 5 Course of lameness grade (score 0–3; median and range) of groups 1 and 2 during the observation period

Horses without synovial structure involvement due to soft tissue trauma also had a highly significant ($p < 0.0001$; related to the $\log_{10}$ values) increase in their mean SAA concentration between admission (range 35.3– 820.5 µg/ml) and 48 hours after surgery (range 3–4156 µg/ml). Similar to G1, a decrease in the SAA concentration followed, with 50% of the horses in G2 taking 11 days (95% CI = 9–14 days) to reach baseline values (<2.7 µg/ml). This time span did not differ significantly ($p = 0.115$; related to the $\log_{10}$ values) between groups. The course of SAA concentrations over time revealed no significant differences (global $p = 0.932$; related to the $\log_{10}$ values) between groups. However, the curve of the SAA concentration of horses without synovial involvement was flatter and had a lower peak at 48 hours than that of horses with synovial structure infection.

Clinical variables

Body temperature patterns yielded no significant differences (global $p = 0.1453$) between groups over time. Body temperature was highest in both groups at admission, with mean values just above the normal range in G1 (38.1°C) and within the normal range in G2 (38.0°C). In the following days, the temperature was within the normal range at all time points in both groups (Figure 4). In contrast, the lameness grade revealed a significant difference between groups ($p < 0.0001$). At the time of admission, horses with synovial involvement (G1) showed moderate lameness (score 2), which improved to a sound status (score 0; Figure 5) within 48 hours of surgical therapy. Horses without synovial structure involvement (G2) initially exhibited only mild lameness (score 1), which resolved to a sound status (score 0) within 24 hours. In the remaining time, the horses remained sound (Figure 5). In horses with synovial involvement (G1), SAA concentrations were positively correlated with body temperature at time points 0 hours ($p = 0.04$) and 24 hours ($p = 0.03$; Figure 6) and with lameness at time points 48 hours ($p = 0.04$), 4 days ($p = 0.04$), 8 days ($p = 0.03$) and 12 days ($p = 0.02$; Figure 6).

Long-term outcome

Fifty-four out of 55 horses (98%) survived to discharge.

In G1, 77% (20 horses) returned to preinjury performance or a higher level of function. Fifteen percent (four horses) were sound but did not return to their previous performance level. One horse still had problems with aseptic synovitis 6 months after

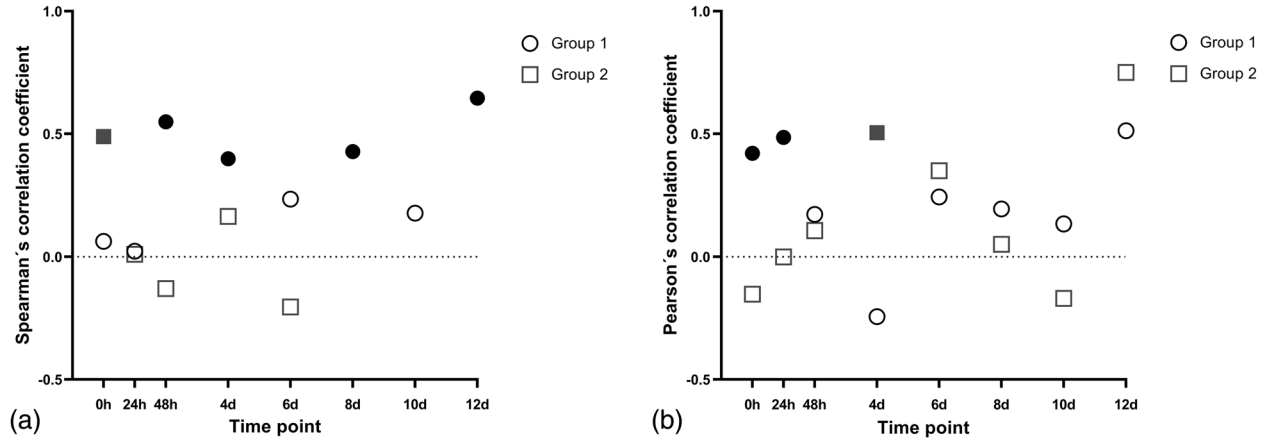

FIGURE 6 (a) Spearman's correlation coefficient (r_s) between serum amyloid A (SAA) concentrations and lameness for both groups and for each time point during the observation period. (b) Pearson's correlation coefficient (r) between serum SAA concentrations and body temperature for both groups and for each time point during the observation period. Filled symbols (■/●) indicate a significant difference ($p < 0.05$) between groups, and empty symbols (□/○) indicate no significant difference ($p > 0.05$) between groups

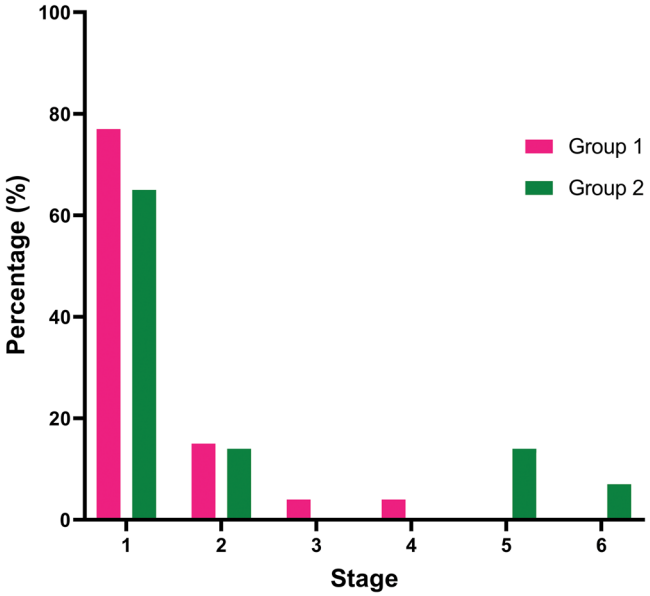

FIGURE 7 Long-term outcome with proportion of horses in percentage for each stage (1–6)

discharge; however, the lameness resolved without further treatment. In G2, two horses were lost to follow-up. Of the remaining horses with soft tissue trauma without synovial structure involvement, 65% (19 horses) were considered to have returned to either their previous or a higher performance level, and 14% (four horses) were considered to be performing at a lower level. Another 14% (four horses) were retired or euthanised due to unrelated conditions (Figure 7).

DISCUSSION

The present study analysed the application of SAA as a systemic biomarker in disease monitoring and therapy adaptation in horses with synovial structure infection due to acute wounds in the limb region and compared this tool with standard clinical variables. In this study, synovial infection produced an SAA response with SAA concentrations following the typical rise-and-fall pattern as already described elsewhere.[14,15,25] SAA concentrations increased immediately and reached an eightfold higher peak than the initial concentration after 48 hours, followed by a continuous decrease until levels reached baseline values after 12 days. In other studies, SAA serum concentrations showed a significantly higher increase in septic synovitis than in aseptic diseases.[13,15,25,34,35] Our results support this, as despite high interindividual differences, horses with synovial structure infection presented a higher concentration curve (with a peak value of 1517.3 µg/ml) compared with those without synovial structure infection (with a peak value of 820.5 µg/ml) (Figure 2). These values are comparable to those observed in related studies.[13,14] Deviations from the typical pattern with a second increase in SAA concentration indicate a recurrence of disease,[14,24] which was confirmed with the three cases presented in this study (Figure 3). In the case of renewed increases in concentrations, re-evaluation of the inflammatory status within the affected synovial structure in terms of bandage change, assessment of the clinical criteria and SF analysis are advised. Thus, SAA levels guide the need for synoviocentesis. However, care should be taken to ensure that the re-increase in SAA levels is not caused by another concurrent inflammatory process, such as pneumonia or thrombophlebitis, which leads simultaneously to an increase in SAA concentrations and makes the interpretation of SAA values challenging or even impossible.[29,36] In our study, SAA courses revealed no statistically significant difference between horses with and without synovial structure infection. This result underlines that, due to its high sensitivity, SAA shows a very non-specific reaction to any inflammatory stimulus.[17,29] The amount of tissue trauma, contributing to the severity of the inflammatory reaction, may possibly have influenced the SAA levels.[24,26,29] The effect remains unclear; however, it is

impossible to standardise because of the clinical character of this study. In addition, the effect of GA on SAA serum levels remains undetermined, as one study reported no increase in SAA concentrations after GA,[30] and yet another study revealed a significant increase in SAA values after GA without surgery.[37] However, GA did not seem to influence the results of this study, as there was no significant difference between groups including horses that were treated under GA as well as standing sedation.

The advantage of SAA compared to fibrinogen, haptoglobin or WBC is the fast response time.[22,26,38] Furthermore, SAA can detect subclinical inflammatory reactions, especially when clinical variables are unclear or even within normal ranges. The severity of the inflammatory reaction depends on the amount of bacteria infecting the synovial cavity.[39] In cases of low-grade contamination, septic arthritis does not necessarily need to be accompanied by fever, which is highlighted by a discontinuous correlation of SAA with rectal temperature (Figure 6). Lameness has proven to be an important clinical variable in monitoring synovial infection postoperatively.[4,13] The results of our study support the evidence that the most reliable clinical variable is lameness, as lameness is positively correlated with SAA concentrations at 48 hours and 12 days, representing the peak time points (Figure 6) and revealing a significant difference between groups. Nevertheless, our three cases of recurrence illustrate that SAA concentrations act more rapidly and more clearly than clinical variables. In addition, clinical variables could have been affected by NSAIDs. The influence of NSAIDs on SAA levels in horses is unclear, as there are no suitable investigations available. In goats and calves, NSAIDs have no effect on SAA concentrations[40,41]; however, Stack et al.[42] found that premedicated horses showed lower SAA levels than untreated horses. However, all the horses in our study received NSAIDs. A major advantage of systemic SAA in monitoring synovial sepsis is that it is not influenced by repeated lavages, repeated synoviocentesis or intrasynovial medication, whereas synovial parameters such as TP, TNCC and %*N* have been shown to increase.[19–21] The use of systemic SAA in monitoring synovial infection due to acute wounds in the limb region improved the outcome in our study, with 77% of horses in G1 returning to preinjury performance or a higher level of function compared to other studies.[4,11,43]

It is possible that all three cases developed recurrent synovial infection around days 10–12 because within this time period, the bacterial growth of the remaining synovial microorganisms could lead to clinical synovial sepsis. Systemic antimicrobial treatment plays a central role in treating contaminated synovial structures. The results of this study show that antimicrobial treatment should be performed over a sufficiently defined time frame and individually for each horse. Our idea was to adapt the duration of antimicrobial treatment to the current inflammatory reaction guided by systemic SAA levels, as bacteria trigger the inflammatory reaction.[2,4,7] The excellent outcome obtained in this study highlights the success of the approach, with 54 out of 55 horses discharged from hospital in a sound state with SAA at baseline values and clinical signs of infection resolved. The recurrence of synovial infection in the three cases correlated with the discontinued antimicrobial treatment. This highlights the importance of continuing antimicrobial treatment at least until SAA concentrations reach baseline levels. Further studies are necessary to evaluate this. A delayed decrease in SAA concentrations shows a lack of treatment success,[14,24,26,29,30,32] highlighting the need to re-evaluate the treatment regimen. Within the initial selection, it should be noted that cefquinome and marbofloxacin are not acceptable first-line antimicrobials.

One limitation of this study is the small sample size due to the restriction to acute wounds occurring less than 24 hours before admission. The study results may also have been affected by the heterogeneity within each group and between the groups, as this was a clinical trial and wounds were not standardised. However, the results can be transferred to a routine clinical setting. In our patient population, it was not possible to create a control group. Due to the clinical design, data obtained per horse could not be standardised more stringently. In addition, returning the horse to the intended performance level was subjected to the owner's decision, and thus, the outcome could have been affected by owner bias.

SAA provides a reliable, useful and rapidly measured parameter to monitor the disease course and to adapt the treatment regimen. Local inflammatory activity within the synovial structure can be assessed based on systemic SAA levels during the healing process. Sequential, repeated measurements of serum SAA concentrations provide an objective tool and improved the outcome of this study compared to other studies.[4,11,43] SAA should thus be routinely used in the postoperative management of horses with synovial structure infection due to wounds in the limb region. Treatment should be adapted to SAA levels in correlation with clinical variables, as SAA offers rapid support in decisions on whether to perform a second surgery or adapt the duration of systemic antimicrobial treatment. As systemic SAA is positively correlated with synovial parameters in the initial synoviocentesis of contaminated synovial structures,[44] SAA should be used in postoperative estimation of the synovial status. However, Synoviocentesis remains the most definitive means for diagnosing synovial sepsis.

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CONFLICTS OF INTEREST

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

AUTHOR CONTRIBUTIONS

Conception of the study and complete study design, study execution and data collection, data analysis, interpretation of data, primary author of the manuscript, and accountable for all aspects of the work, final approval of the version to be published: Anke-Charlotte Müller. *Data analysis and interpretation of data, power analysis, revising the manuscript critically for important intellectual content, final approval of the version to be published*: Kathrin Büttner. *Substantial contribution to conception of the study, overall supervision in study design, interpretation of data, oversight in drafting the article, accountable for all aspects of the work, final approval of the version to be published*: Michael Röcken.

ETHICS STATEMENT

The study was reviewed and approved by the ethics committee of the regional council Giessen, Hessen, Germany and was not considered as an animal experiment (reference number kTV 6-2018). All blood samples were collected during routine diagnostic work-up of the cases. Owners gave their informed consent to use the results for the research purposes of this study.

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DATA AVAILABILITY STATEMENT

The dataset is available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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