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Retrospective Analysis of the feasibility of serial plasma SAA measurements as a monitoring tool for treatment response of horses that underwent surgery due to septic synovitis

Diploma Thesis

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Contents

1 Introduction

Septic synovitis is a severe, common disease in horses. Immediate, appropriate diagnosis and treatment is utterly important for the patients' survival and future career. The sooner the condition is correctly diagnosed and appropriately treated the better are the horses' chances not only for survival but also for returning to prior athletic performance. (Lugo and Gaughan 2006) This study therefore focuses on the acute phase protein Serum amyloid A (SAA) as additional tool for earlier diagnosis and better monitoring of septic synovitis.

1.1 Septic synovitis

Infections of a bursa, tendon sheath or joint may happen to any horse irrespective of race, age, or sex. In this segment, the current scientific status concerning etiology, clinical findings, diagnosis, therapy, and prognosis is summarized.

1.1.1 Etiology

In adult horses, septic synovitis can be caused by wounds penetrating synovial structures, by perisynovial wounds that secondarily affect adjacent synovial structures, by hematogenous spread or, iatrogenic via synoviocentesis or surgical intervention. (Meijer et al. 2000, Morton 2005)

Foals are most commonly affected by septic arthritis due to bacteremia induced by failure of passive transfer of colostral immunoglobulins. Other risk factors are delayed time to stand and suckle, prematurity, dysmaturity, dystocia and peripartum disease of the mare. (Annear et al. 2011, Meijer et al. 2000) Common ways for bacteria to enter the patient are hereby the umbilicus, the respiratory and gastrointestinal tract. (Annear et al. 2011) Because of the differences in etiology and therapy, foals are not further discussed within this diploma thesis.

Wounds at the distal part of the adults' horse extremity especially run the risk of affecting a synovial cavity due to the sparse soft tissue cover and the high number of synovial structures located in this area. Synovial infection is caused either by bacteria, organic and/or by anorganic material (foreign bodies) that entered the synovial structure. (Ludwig and van Harreveld 2018)

Most commonly in wounds as well as in iatrogenic infected synovial cavities, ubiquitous gram-positive bacteria like Staphylococcaceae and Streptococcaceae are responsible for the septic process. Gram-negative bacteria (Enterobacteriaceae, Enterococcus spp., Pseudomonas aeruginosa spp…) are also isolated in some cases and were found to correlate with poor prognosis for survival of patients, especially if multi drug resistant strains were isolated.

Gilbertie et al. (2018) found a 119.24 higher chance not to survive septic synovitis, if gram-negative bacteria or multi-drug resistant bacteria were involved (OR 119.24, 70.57–201.46, p < .0001). (Gilbertie et al. 2018)

Iatrogenic caused septic synovitis due to intra-synovial medication or surgery can be especially challenging. Intra-synovially instilled corticosteroids or glycosaminoglycans inhibit the local synovial defense mechanisms, may lead to a delayed diagnosis and therefore longer/more severe course of disease. (Morton 2005, Schneider et al. 1992, Smith et al. 2019)

1.1.2 Pathophysiology of septic synovitis

Depending on the type and number of microorganisms involved, the state of the affected synovial cavity and the immune system of the patient, severe infection takes place or can be stopped by natural defense mechanisms of the synovial structure (e.g.phagocytosis, cytokines,…) (Ludwig and van Harreveld 2018, Morton 2005).

An inflammatory reaction starts as antigens of microorganisms, foreign or organic material enter the synovial cavity and are recognized by the immune system. Neutrophil granulocytes (neutrophils) as part of the immediate and innate immune system are the first cells that arrive to eliminate the infection. As neutrophils phagocytize they produce free radicals and release lysozymes, collagenases and cytokines, such as Interleukin-1beta (IL-1β) and tumor necrosis factor alpha (TNF-α). (Ludwig and van Harreveld 2018, Morton 2005)

Due to synovial membrane hyperemia resulting in the disruption of blood-synovial barrier not only more neutrophils but also macrophages and more inflammatory mediators can invade the synovial cavity, augmenting the inflammatory reaction by activating for example plasmin (amongst others, enzyme for fibrinolysis) and kinin (increasing vascular permeability, pain perception, etc.) pathways. All these processes together lead to further release of inflammatory cytokines by chondrocytes and synoviocytes and subsequently to a change in cell-metabolism: More matrix metalloproteinases are released; less proteoglycans are produced. (Morton 2005)

In the course of septic synovitis, the synovial membrane plays an important role as a reservoir for pathogens because microorganisms tend to colonize it and therefore treatment (antimicrobials) may not reach this site of infection appropriately. As the disease progresses the reduced proteoglycan production and increased collagenase activities (MMPs) lead to destruction of cartilage matrix and subsequently to irreversible damage to the joint. Furthermore, fibrin – present in a high amount in longer lasting septic joints – is deposited during the course of disease. Fibrin within the cavity not only forms a reservoir for

microorganisms, but it also hinders the normal course of motions in changing synovial fluid flow and joint homeostasis. (van Weeren 2016)

Additional to fibrin, pain and swelling disturb normal mobility. Moreover, joint or tendon sheath effusion increases pressure within synovial structures and affects perfusion of the vascularized areas, leading to more pain due to ischemia, further damage and dysfunction. (van Weeren 2016)

The more fibrin reaches the area the more it tends to aggregate and forms a fibrinocellular conglomerate, the so-called pannus. Pannus disturbs motion even more, builds a matrix in which microorganisms, necrotic tissue or foreign materials are embedded making them even harder to remove and reach with systemic antimicrobials. (Ludwig and van Harreveld 2018)

In tendon sheaths and bursae, compared to joints, the described pathophysiological process results in fibrosis and adhesion of the tendon within the sheath/bursa resulting in chronic lameness and reduced range of motion. (Schneider et al. 1992)

In summary, the pathophysiology of septic synovitis is a complex interaction of the immune system with microorganisms, necrotic tissue or foreign material, which ultimately leads to a massive inflammatory reaction and disbalances of anabolic and catabolic processes within the synovial structure causing irreparable damage to joints, tendon sheaths or bursae.

1.1.3 Clinical findings

Common clinical signs are distension, swelling, pain and reduced mobility of the affected joints, tendon sheaths, or bursae. Many horses are presented with high grade or even non-weight-bearing lameness. (van Weeren 2016)

The patients' history is very important, to determine the duration of the condition and, whether a septic synovitis is present. Especially recent wounds or injections indicate a possible involvement of synovial structures. Septic arthritis caused by hematogenous spread of bacteria is not very common in adult horses, but still a possible differential diagnosis (approx. 0.9 % of horses >6 month old) and should therefore be considered (Schneider et al. 1992). Different conditions may obscure the clinical findings.

In some cases, the original trauma may seem worse enough to cause severe lameness, and an infection may be missed. In other cases, wounds that provide synovial drainage, release tension and relieve the horses' pain, resulting in a milder lameness than expected in septic synovitis. Furthermore, horses with recently treated injuries may be almost sound on initial

examination, and their condition may be underestimated. (Ludwig and van Harreveld 2018, van Weeren 2016)

In any case, a thorough physical examination of the patient is crucial. Vital parameters may vary from normal to increased heart and respiratory rates and rectal temperatures higher than 38 °C. Areas in which wounds are expected should be clipped and thoroughly examined. Typical signs of inflammation, such as perisynovial soft tissue heat and swelling, synovial structure effusion and sensitivity to palpation and manipulation are seen in horses with septic synovitis. (Bertone and Cohen 2011, Ludwig and van Harreveld 2018)

1.1.4 Diagnosis

Since it takes several days for leucocyte count, total protein and plasma fibrinogen to increase significantly within blood samples (in comparison to healthy horses), septic synovitis is diagnosed by synovial fluid analysis (Bertone and Cohen 2011). In experimental studies, synovial fluid changes within 24h after infection (Bertone et al. 1987). In any suspicious case, synoviocentesis should be performed and in patients with wounds in close proximity to a synovial structure a positive pressure tests can give further information. Bacterial culture and radiologic imaging are further important diagnostic methods that are frequently used in those cases.

A non inflamed and, more important, not infected appropriate area for synoviocentesis should be located, clipped thoroughly, and prepared aseptically. Afterwards synoviocentesis is performed and synovial fluid is collected in Ethylenediaminetetraacetic acid (EDTA) tubes and bacterial culture vessels. In some severe cases, or in cases with synovial run off through a penetrating injury, only very little amounts of fluid can be obtained (due to fibrin clots or pannus). In these cases, a cytological smear can be prepared, or sterile saline solution can be injected and aspirated. In this diluted samples, macroscopic examination and cell count outcome are not reliable. To diagnose communication (positive synovial pressure test) of a wound with a synovial structure, 50-200 ml balanced, sterile saline solution is injected after fluid collection, to test leakage through the wound. Afterwards, antimicrobials are instilled. (Bertone and Cohen 2011)

1.1.4.1 Synovial fluid analysis

Analysis of synovial fluid starts macroscopically. Normal synovial fluid appears lightly yellow, clear, and very viscous. Infected synovial fluid may differ in all three attributes. It can look

normal to dark orange or even red, cloudy, and less viscous (due to less produced hyaluronic acid within inflammatory synovio- and chondrocytes).

Normal cell counts are less than 500 cells/mm^3 or µl, septic joints for example exhibit 30 000 – 100 000 cells/mm^3. Also, instead of monocytes and macrophages, most white blood cells in infected synovia are neutrophils, to be specific up to >95 %. Total protein may rise up to 4 – 5 g/dL (reference value < 2 g/dL). (van Weeren 2016)

1.1.4.2 Bacterial cultures

Additional to synovial fluid analysis, the preparation of a bacterial culture is recommended. In some cases, synovial fluid smears show bacteria, which are stained with haematoxylin and eosin and/or gram staining. In most cases, for detection of bacteria, vials with blood culture medium are needed. Unfortunately, bacteria can only be isolated in approximately 50 % of the cases. (Gilbertie et al. 2018, Ludwig and van Harreveld 2018, van Weeren 2016)

1.1.5 Radiological Imaging

Radiologic imaging is of special interest in foals and also essential in adult horses to determine whether the septic process is accompanied by septic osteomyelitis, fractures, osteitis, osteoarthritis or – considering especially foals – physitis. Moreover, contrast radiography can be used to assist in diagnosing communication of a wound with the affected synovial structure and in detecting cartilage injuries. (Bertone and Cohen 2011, Morton 2005)

As computed tomography (CT) shows bony structures better than magnet resonance imaging (MRI), and conversely, MRI soft tissues better than CT, contrast CT's were tested to enhance the diagnostic power of CT (Pauwels et al. 2021, Suarez Sanchez-Andrade et al. 2018). In one study CT-arthrography showed a high sensitivity for detection of cartilage injuries compared to MRI-arthrography, classic CT and MRI (Suarez Sanchez-Andrade et al. 2018). However, these methods are relatively expensive – and therefore rarely used. (van Weeren 2016)

Most commonly no severe changes are seen in radiographs but sometimes air within the synovial cavity is found, underlining communication of wounds with synovial structures. Bone lysis seen in radiographs suggests bone infection (osteitis, osteomyelitis) and therefore negatively affects prognosis. (Ludwig and van Harreveld 2018)

Ultrasonographic imaging is also recommended for further diagnosis. Typical findings are synovial effusion, thickening of synovial membrane, intrasynovial fibrin, echogenic synovial fluid and focal hyperechogenic areas. (Beccati et al. 2015)

1.1.6 Treatments

1.1.6.1 Surgery

Inflammation and microbes have a severe negative impact on the affected synovial cavities as described earlier. The longer the conditions persist, the worse the damage. Additionally, to decreased pH within synovial fluid, medications for example, aminoglycosides, show reduced activity. Cell detritus and inflammatory mediators as well as catabolic enzymes affect proteoglycan levels and will eventually cause irreversible damage to the cartilage collagen network. (van Weeren 2016)

Those reasons explain the need to act fast. In best case scenarios, the joint, tendon sheath or bursa is treated (lavaged) before inflammation started. There are at least three ways to perform a synovial lavage in horses:

- Through and through lavage (hypodermic cannulas)
- Arthrotomy
- Arthroscopy

Through-and-through lavage is the least invasive one. It can be performed standing under sedation and local anesthesia or general anesthesia using at least two needles. The big disadvantage is that with this method, the surgeon works blindly and the structure cannot be evaluated for pathological changes (e.g., cartilage damage) and usually pannus within the structure cannot be removed. For the lavage, at least 6 – 7 l of sterile solution or ringer solution are instilled.

Arthrotomy and arthroscopy both have the advantage of visualizing the cavity and cell detritus, fibrin, pannus, foreign bodies, and necrotic tissue can be removed. Studies showed that arthrotomy eliminates joint infection faster than arthroscopy but is more invasive and more likely to lead to complications (ascending infection). That is why, nowadays arthroscopic lavage is the technique of choice. (van Weeren 2016)

Additionally, to arthroscopic lavage a particular ultrasonographic technique has been used simultaneously, to break adhesions of and disrupt bacteria on synovial structures via acoustic cavitation. This method is not used in horses yet, but one case report revealed promising results. (Rinnovati et al. 2020)

1.1.6.2 Antibiotics

After septic synovitis is diagnosed, antimicrobials should be administered immediately. Because bacterial culture takes a few days to grow and bacteria can only be isolated in about 50 % of affected horses, it is usually not possible to instantly apply a microorganism-specific antimicrobial treatment. Therefore, broad-spectrum antibiotics are used, until results of the antimicrobial susceptibility test are available. For Gram-positive and Gram-negative bacteria, β-lactam antibiotics and aminoglycosides are used. In extremely contaminated wounds, where anaerobes are expected, metronidazole may be added. Additional to systemic administration, local application of antibiotics is a common practice. With local administration higher local concentrations can be achieved without increased risk for side effects. (van Weeren 2016)

There are different approaches for local antimicrobial treatment: intrasynovial application, intravenous regional limb perfusion and intraosseus perfusion.

Intravenous regional limb perfusion (IVRLP) works through intravenous application of antibiotics near by the affected synovial cavity. To stop antibiotics from distributing into systemic circulation, a rubber or pneumatic tourniquet is applied proximal to the puncture site. Poor blood supply at the distal proportions of the limbs makes systemic antibiotics often not as sufficient as IVRLP. A further advantage is the high-pressure concentrations between intra- and extravascular compartments that are obtained during this procedure. Which is why studies show higher concentration and longer disposition of the antibiotic within the area they are needed. Most common used veins are the palmar digital, saphenous and cephalic veins. The ideal amount of instilled antibiotics varies between studies. 20 – 250 ml volumes were tested by different study groups, some found no significant differences between applied volumes, while others did find 100 ml perfusate resulting in higher concentrations of antibiotics within the desired structure, than 30 – 60 ml of the same perfusate. (Kelmer 2016, Oreff et al. 2016) However, amongst others due to better compliance of the patients usually 30 ml of perfusate are instilled in digital and 60 ml in cephalic and saphenic veins. To treat the veins with care, small gauge butterfly catheters are recommended. (van Weeren 2016)

Antimicrobials of choice are aminoglycosides as amikacin and gentamicin, due to their concentration dependency. Also, ceftiofur, vancomycin, erythromycin, enrofloxacin, marbofloxacin and chloramphenicol have been effective, due to their ability to stay a long time above the minimum inhibitory concentration (MIC) (wanted are approx. 10 times > MIC in

IVRLP) and because they maintain a high maximum of concentration during the IVRLP. (Kelmer 2016)

Intraosseous limb perfusion was also evaluated (in cases with additional osteomyelitis) but so far showed, no advantages over intravenous regional limb perfusion, due to higher complication rates (33 % in intraosseous limb perfusion and 12 % in intravenous regional limb perfusion). (Rubio-Martínez et al. 2012, van Weeren 2016)

1.1.6.3 Pain management

First step of reducing the pain is thorough lavage. Phenylbutazone and Flunixin are the NSAIDs of choice, while fibrocoxib and meloxicam can also be used. (Ludwig and van Harreveld 2018)

In more severe cases multimodal pain management using opioids systemically or locally together with local anesthetics or alpha 2 agonists (e.g. epidurally) should be considered. (Ludwig and van Harreveld 2018)

1.1.7 Further arrangements

As the patient with synovial sepsis is an emergency, stall confinement is obligatory during the acute phase. In addition, regular bandage changes need to be done to reevaluate wounds and arthroscopic portals. Physiotherapy is recommended if the patient improves. In cases with severe joint damage, surgical ankylosis may be a way to further improve the patient's well-being. (van Weeren 2016)

1.1.8 Prognosis

Septic synovitis is a serious condition, but due to treatment-adaption in the last decades (fast, arthroscopy, IVRLP) horses have – depending on authors – an 80-85 % chance of survival to discharge from the hospital and 33-77 % of these horses return to full athletic performance. (Ludwig and van Harreveld 2018, van Weeren 2016)

The percentages vary due to different occurrence of septic synovitis. Involved structures, bacteria and duration of infection are important prognostic factors.

When treated within 24h with local and systemic antibiotics, thorough lavage and debridement, horses have the best outcomes and lowest infection rates. (Gibson et al. 1989, Joyce 2007, Schneider et al. 1992) In horses with septic arthritis studies showed 54 % and 85 % chance of survival (Gibson et al. 1989, Schneider et al. 1992). For septic tenosynovitis the chances for survival were better with 78 % and 90 % in different study groups (Frees et al. 2002, Honnas

et al. 1991). The return to full performance differs from 56 % with Honnas et al. (1991) and 70 % in the research work of Frees et al. (2002) septic bursitis has lower (67 %) survival rates compared to tenosynovitis. In cases where the tuber calcanei is involved prognosis is even worse with only 44 % chance of survival (Post et al. 2003). Navicular bursitis also offers guarded prognosis due to involvement of the insertion of the deep digital flexor tendon and the adjacent navicular bone. Immediate endoscopic treatment and antimicrobial therapy is crucial for good survival rates. (Joyce 2007, Wright et al. 1999)

Different types of bacteria also alter the prognosis for septic synovitis. Compared to all other bacterial infections in one study with 205 horses, enterobacteriaceae infection resulted in the highest euthanasia rates. The chances of non-survival were also increased when coagulase positive staphylococcus spp., β-hemolytic streptococcus spp., enterococcus spp., pseudomonas aeruginosa or other gram-negative species were involved. Best prognostic factor for survival and earlier discharge from hospital in this study was synovial fluid culture that did not show growth of any kind of bacteria. (Gilbertie et al. 2018)

Duration of a condition also affects prognosis. Treatments starting within 24 hours enhance the chances of survival, return to full performance and reduce the risk of developing septic arthritis. The longer the infection remains untreated, the more pannus accumulates, and the higher the risk for developing osteochondral lesions and osteomyelitis. (Gibson et al. 1989)

1.2 Serum Amyloid A (SAA)

The earlier septic synovitis is diagnosed, the better are the chances of the horse to recover completely. Early detection and treatment are obligatory. Since white blood cell (WBC) count and the acute phase protein (APP) fibrinogen need time to increase and illustrate changes in terms of inflammation, other markers were investigated that detect inflammatory reactions more reliable. (Long and Nolen-Walston 2020)

1.2.1 Acute phase proteins

Detection of inflammation not only in horses but in humans, dogs, cats, …etc. is successfully done via acute phase proteins. Acute phase proteins are synthesized during an acute phase reaction by hepatocytes triggered by inflammatory mediators and cytokines released by immune cells, such as TNFα and IL1β. (Mealey and Long 2018)

There are different types of APP's classified according to their in/decrease during an inflammatory process. There are minor and moderate APPs, that increase 1 to 10-fold from

the original concentration in blood samples and negative APP that decrease during the APR. (Jacobsen and Andersen 2007)

Major acute phase proteins are defined as proteins produced in the liver that have very low or even undetectable concentrations in healthy patients but rise rapidly up to 1000-fold once an inflammatory process occurs. (Long and Nolen-Walston 2020)

In horses and cats Serum amyloid A is considered as the major acute phase protein, in dogs and humans, C-reactive protein (CRP) is used.

1.2.2 SAA

Serum Amyloid A, the only major acute phase protein in horses is produced during the acute phase reaction (APR) by the innate immune system after nearly any kind of tissue injury (e.g. inflammation, infection, colic, after surgery, etc.).

SAA circulates in complex with high-density lipoproteins and is therefore classified as a 9-11 kilodalton apoprotein. (Witkowska-Piłaszewicz et al. 2019)

As discussed earlier, damaged tissue and cells release molecules, that activate immune cells: macrophages and monocytes. These in turn produce inflammatory mediators such as cytokines (IL-1, TNFα, IL-6) that stimulate paracrine and endocrine immune pathways causing signs of inflammation and hepatic cells to synthesise APP's. (Jacobsen and Andersen 2007)

SAA is also released as extrahepatic isoforms by cells with contact to the external environment such as the gastro-intestinal tract, the mammary glands, and the airways. Furthermore SAA is synthesised by chondrocytes during infections and released into the synovial fluid. (Witkowska-Piłaszewicz et al. 2019)

1.2.2.1 SAA and healthy horses

In healthy horses SAA values measure between 0 – 20 mg/L but mostly < 1 mg/L. Differences of SAA levels have been found in healthy pregnant mares one week before parturition in one study but could not be confirmed in other studies. (Witkowska-Piłaszewicz et al. 2019)

Athletic performance, e.g. long distance rides (120-160km) can also lead to a significant increase in SAA levels up to 10-fold in healthy horses (Cywińska et al. 2012). However, levels measured after performance still stayed much lower than SAA levels during an acute inflammation.

1.2.2.2 SAA and inflammation in horses

SAA as the major acute phase protein in horses, starts to increase about 6h after the beginning of an inflammatory reaction and peaks up to 1000-fold, 24h – 48h later. Additionally, it decreases rapidly within 12h after inflammation resolved. Because fibrinogen only increases moderately (only 1 to 2-fold), the clinician may miss mild tissue damages, which is why SAA measurements are seen as a more reliable choice for detection of inflammation in such cases. (Long and Nolen-Walston 2020)

SAA as a marker of inflammation was and is investigated for many different diseases in equine medicine.

In neonates SAA is suggested to help the differentiation of systemic inflammation from local processes as – for example – umbilical abscesses. Therefore, SAA measurements can add in speeding-up therapeutic measures and potentially increase survival rates in foals.

Investigations in reproductive medicine found that mares that had acute pyometra, septic placentitis and endometritis, had significantly higher SAA values compared to those with only inflammatory lesions. There have been studies that not only show an increase in SAA but also correlations between dose of pathogens (E.coli) and level of SAA (the more, the higher), in induced endometritis. Subclinical endometritis and chronic placentitis could not to be detected using SAA measurements.

In Internal medicine, studies showed SAA values as high as 1000 mg/L in horses infected with Streptococcus equi subspecies zooepidemicus, whereas SAA only increased to 100 mg/L within different viral infections. Viral infections showed no positive correlation considering higher doses of virus and SAA levels, contrary to findings in artificially induced endometritis. Considering the respiratory tract, SAA can be used for differentiation of bacterial infection (or diseases with similar clinical signs) and non-infectious diseases. There are also studies concerning gastro-intestinal issues. Colitis, enterocolitis, abscessation and peritonitis have shown increased SAA values, whereas strangulated or non-strangulated obstructions have not, which may also help with further diagnostic and therapeutic decisions. (Witkowska-Piłaszewicz et al. 2019)

1.2.2.3 SAA levels of horses with septic synovitis

In arthritis, tenosynovitis and bursitis, SAA levels may also be used to distinguish synovial inflammation from synovial sepsis. High SAA levels – as mentioned – up to 1000-fold of baselines of healthy horses (≤ 20 mg/L), are indicative for septic synovitis. (Witkowska-Piłaszewicz et al. 2019)

Limitations of SAA detecting inflammation are measurements early in the disease process (<12h), low grade inflammation/infection and well sequestered disease processes. (Haltmayer et al. 2017)

The definite diagnosis and the monitoring of septic synovitis is usually determined by repeated synoviocentesis of the affected structure as mentioned earlier. However, several studies suggest the difficulty and risk of repeated synoviocentesis for diagnostic purpose (e.g. reliability after intraarticular medical treatment). (Yoshimura et al. 2020)

2 Aim of the study

Studies showed that arthroscopy, through and through lavage and local amikacin-applications change synovial fluid parameters - especially TNCC and TP – and potentially lead to inconclusive synovial fluid analysis (Long and Nolen-Walston 2020). Synoviocentesis and collection of synovial samples can be difficult, especially under field conditions. Fibrin formation, synovial hypertrophy, and synovial run-off through an open wound can prevent successful collection of synovial fluid samples (Bertone and Cohen 2011, Haltmayer et al. 2017).

Furthermore, every synoviocentesis performed on a patient, bears the risk of reinfection and worsening of the situation.

Having a stable, reliable systemic marker for monitoring septic synovitis in horses, such as Serum amyloid A, would overcome these disadvantages and therefore improve medical care for these patients.

The aim of this retrospective study is therefore to evaluate if serial systemic SAA measurements are feasible as a monitoring tool for treatment success in horses with septic synovitis in a clinical setting.

3 Materials and Methods

3.1 Design

We collected data of horses that underwent arthroscopy due to septic synovitis at the Veterinary University of Vienna in the years of 2015 – 2020, out of the Universities software system, the Tierspitalsinformationssystem (TIS). Accordingly, the present study can be regarded as a retrospective monocentric analysis.

3.2 Collective of patients

The following inclusion and exclusion criteria were to be considered as relevant for study participation.

Horses to be included had to:

- be older than one year of age
- undergo arthroscopic lavage due to septic synovitis
- have SAA values taken in regular intervals and more than one time.

Included horses either had to have one or multiple wounds penetrating synovial structures or septic synovial fluid analysis outcomes to receive an arthroscopic lavage.

Additional to horses under one year of age, pregnant mares, horses with systemic illness and horses with further underlying septic processes (such as sepsis) have been excluded from this study.

3.2.1 Medical history

Since septic synovitis is often related to wounds, the age of the injury at the time of admission was recorded wherever possible and categorized in < 24h and ≥ 24h hours. In addition, whenever given, treatment history, especially with intra articular medication, or previous arthroscopic treatments were recorded.

3.2.2 Examinations

After obtaining the medical history, clinicians at the equine hospital, vetmeduni vienna examined the patients clinically and orthopedically. Lameness was classified in 5 grades at walk. (Edinger et al. 2014)

Horses that were lame at walk or had obvious swelling or wounds, did not receive further orthopedic examination as flexion tests, diagnostic anesthesia, or evaluation at trot.

For further information about the horses' condition, blood samples were taken either through a venous catheter they received for surgery, or through venipuncture with a vacutainer system (Vacuette, Greiner Bio-One GmbH, Frickenhausen, Germany). After collection in ethylenediaminetetraacetate (EDTA) and heparinized tubes (Vacuette, Greiner Bio-One GmbH, Frickenhausen, Germany) the universities central laboratory analyzed the blood. A complete blood count (out of EDTA-tubes) was performed and creatinine, total protein glutamate dehydrogenase, gamma-glutamyl transferase, creatinine kinase, potassium, and fibrinogen (out of heparinized plasma) was determined in all horses. Furthermore, SAA values of all horses were taken either at admission or on the next day, depending on the clinician in charge and availability of laboratory equipment during emergency services. Then, the measures where repeated approximately every 48h until the patient was released from the hospital.
SAA was measured using heparinized plasma at the Central Laboratory of the Veterinary University of Vienna, Austria. The samples were processed with immuneturbidimetry (LZ test SAA, Eiken Chemical Co,Tokyo, Japan). Results <10 mg/L were deemed to be physiologic. (Jacobsen et al. 2006, Swancar-Haid 2011)

Before surgery, synoviocentesis was performed and horses were diagnosed with septic synovitis or communication of a wound with a synovial structure, through the assessment of synovial fluid, smears, cell counts and/or by performing a positive pressure test. Because of complications concerning the collection of synovia in diseased structures (fibrinous flocculation may handicap the sample collection via the small lumen of the needle), in some of the cases it was necessary to instill sterile saline solution into the synovial structure and subsequently aspirate diluted synovial material to obtain a sample for analysis.

Synovial smears were evaluated after staining (checking the morphology of the cells, the quality of mucin precipitate and the possible presence of bacteria) by, either the clinicians themselves or - depending on availability/working hours - the Universities' Central Laboratory Unit.

3.2.3 Techniques of sample evaluations

In a first step, the samples were evaluated macroscopically: amount (if undiluted), color and transparency (cloudy, flocculation?) were determined.

Afterwards, the Central Laboratory prepared a smear and a cytospin (if needed) out of each sample they received. The cells were stained with a Romanowsky-type stain (Haemafix™

Biomed Labordiagnostik GmbH, Oberschleißheim, Germany), to evaluate the cell-types and their morphology.

Depending on the constitution of samples different techniques were used to perform cell counts. Cell counts of undiluted samples were performed with a laser-based hematology system (Advia 2120™, Siemens Diagnostics, Erlangen, Germany) to obtain the Total Nuclear Cell Count (TNCC). In diluted samples only the ratios of neutrophils and monocytes were determined, as the total amounts would not be comparable, with those of undiluted ones. To decrease viscosity of some samples, incubation with hyaluronidase powder was performed prior to automated cell counts.

Additional to cell counts and ratios total protein was determined with a refractometer. Total protein deemed normal when it was lower 2.5 g/dL.

3.2.4 Diagnosis and decision for surgery

Diagnosis of septic synovitis and the decision for surgery were determined by the clinicians in charge and based on the clinical and orthopedic examinations combined with the laboratory findings. Consequently, if communications of wounds with synovial structures could be detected, if laboratory examination revealed a TNCC greater 20 x 10^3 / µl, neutrophil granulocytes more than 80 % of the fluid and/or a total protein greater 4 g/dL, horses were recommended to undergo surgery.

3.2.5 Treatment

After the decision for surgery was made by the clinician in charge, all horses received intravenous catheters in either the right or left jugular vein. All horses were treated pre- and postoperatively with broad spectrum antibiotics – penicillin G (30 000 IU/kg, i.v. QID), gentamycin (6.6 mg/kg, i.v. SID) - and nonsteroidal anti-inflammatory drugs (NSAIDs) – flunixin meglumine (1.1 mg/kg, i.v. BID). In cases with positive bacterial culture and subsequent antimicrobial sensitivity testing, antibiotic treatment was adjusted accordingly.

3.2.6 Monitoring after surgery

Horses received SAA measurements, synoviocentesis and clinical examinations to reevaluate their health status. In some cases, further lavages were needed, or pain medication had to be adapted.

3.3 Data management and privacy

The data relevant for the presented study were collected from the database (Tierspitalsinformationssystem, TIS) transferred to an Excel spreadsheet. In a further step, the Excel database was transformed into a corresponding SPSS matrix. The sensitive data was secured on end devices with access restrictions so that only authorized persons had access to it. Compliance with the current data protection guidelines can be regarded as guaranteed; in particular, no conclusions about individual cases can be drawn from the results.

3.4 Ethical aspects

This retrospective study did not need any approval of the ethic commission of the veterinary university of Vienna (Veterinärmedizinische Universität Wien), because no study-associated treatment was performed. All horses were treated based on decisions of their clinician in charge.

3.5 Statistical Analysis

The descriptive and inferential statistical analyses were performed using IBM SPSS® 28 for Windows®. The significance level for the conclusive statistics was set in advance at $\alpha = 5\ \%$, corresponding to the probability of error. Standardized effect sizes were determined according to Cohen's (1988) classification. Values of $r \geq .10$ were referred to as small, $\geq .30$ as medium, and $\geq .50$ as large (Bortz and Döring 2016).

3.5.1 Descriptive Statistics

For descriptive statistics key values were determined. To test normal distribution the Kolmogorov-Smirnov and Shapiro Wilk tests were used.

To minimize the hereby identified skewness of data distribution, with extreme values higher 100-fold typically increased values (1 000 vs 55 000), SAA was lg10-logarithmized for some data analysis.

3.5.2 Inferential statistics

To investigate differences in SAA values between horses with different durations of injury (<24h or ≥24h) and horses receiving one and more than one arthroscopy, the Mann-Whitney-U-Test was used. (Weiß 2013)

For the assessment of changes in SAA levels at admission and SAA levels between different timepoints after surgery, SAA values were compared using the nonparametric Wilcoxon matched pairs rank test. (Weiß 2013)

To describe the relation between number of arthroscopic lavages and SAA values at timepoint 1 (T1, first value post-surgery), Spearman's rank correlation r_s was used. (Weiß 2013)

To compare results of synovial fluid analysis and SAA levels post-surgery, the non-parametric Kruskal-Wallis test was used. (Field 2009)

In a further step, a multiple linear regression model was performed to analyze, possible predicting factors for post-surgical SAA levels. SAA levels were hereby seen as dependent, the predictors (e.g. age, wound age and synovial fluid outcome) as independent variables. Required assumptions (correct scales of measurements in predictors and dependent variables, linear relationship between predictors and dependent variable, no multicollinearity, homoscedasticity, and normal distribution of residuals) were considered and therefore SAA values were logarithmized. Predictors were subjected to model testing using the backward stepwise method. To fit synovial fluid analysis outcomes into the model, dummy codes had to be established, so that the possible manifestations borderline and positive synovial fluid outcome could be compared to nonspecific synovial fluid outcome. (Backhaus et al. 2016)

In another approach a canonical, discriminant analysis was performed. The objective was to assess whether horses can be assigned correctly into the three synovial groups if only their expression of characteristics such as age, wound age (or rather endurance of clinical signs) and SAA level after surgery was known. (Bühl 2012). Wilks-Lambda, as an inverse test value, was used to determine whether the variables in the groups differ significantly. (Backhaus et al. 2016)

4 Results

4.1 Sample

A total of 64 horses met the inclusion criteria. Mean age was 9 (± 5.5) years and gender was evenly distributed.

The data of n = 64 horses, i.e. 26 (40.6 %) geldings, 35 (54.6 %) mares and three (4.7 %) stallions, at the age of 1.2 to 28.1 years (*Md* = 9.3) could be collected. The median age of geldings administered for surgery was 10.6, of mares 9.0 and of stallions 1.8 years as illustrated in Table 1. (also see Appendix 1: Age and sex distribution).

Table 1 Key values of age (years) considering the horses' sex

Sex	N	*M*	±*SD*	min – max	*Md*	IQR
Gelding	26	10.3	5.8	2.2 – 24.5	10.6	4.7 – 14.9
Stallion	3	4.0	4.4	1.2 – 9.1	1.8	1.5 – 5.4
Mare	35	9.3	5.3	2.0 – 28.1	9.0	6.4 – 11.8
Total	64	9.5	5.5	1.2 – 28.1	9.3	4.7 – 13.0

4.1.1 Presentation at admission

In 57 horse’s lameness at admission was recorded. In 30 (52.6 %) of these cases the horses were at least 3/5 lame at walk. From the remaining 27 (47.4 %) less than 3/5 lame horses, 16 (53.3 %) were premedicated with NSAID’s or have been sedated by the referring veterinarian prior to admission at the clinic.

4.1.2 Duration and location of synovitis

In 59 cases the duration of injury prior to admission could be determined and was grouped in < 24h and ≥ 24h. In 28 (47.5 %) patients injuries happened < 24h before admission, in 31 (52.5 %) of 59 horse’s injuries were older than 24h at the time of admission.

In 41 (64.1 %) cases at least one joint was affected, compared to only 13 (20.3 %) treated for tenosynovitis and ten (15.6 %) diagnosed with bursitis. 21 (32.8 %) horses were treated for septic arthritis distal to the carpal or tarsal joint. Whereas in 11 (17.2 %) patients one of the tarsal joints was affected and in the remaining nine (14.1 %) horses, other joints (three elbows, five knees, one carpus). All horses diagnosed with septic tenosynovitis had the issue distal to carpal and tarsal joints. Whereas seven (70.0 %) cases suffered from septic bursitis of the

bursa subtendinea calcanea, two cases (20.0 %) of septic synovitis of the bursa bicipitalis and one (10.0 %) from bursitis of the navicular bursa.

4.1.3 Synovial fluid analysis

Synovial fluid analysis was performed at least once in 63 horses, before surgery. In the remaining horse (n=1) only a pressure test was performed. From these horses, 59 (92.2 %) showed at least one of the following values: increased TNCC > 20 x 10^3 / µl, increased neutrophil granulocyte percentage > 80.0 % and increased total protein > 4 g/dL. Nonspecific outcomes were evaluated in five (7.6 %) cases, due to extraction-related blood-contamination, artefacts within the sample or unspecific cytology. Further or at least two synovial samples were taken from 51 horses. A third synovial fluid analysis was taken from 28 (43.7 %) of all horses. Our data revealed up to seven synovial fluid analyses for two horses that were negative at their final one.

4.1.4 Bacterial culture

Bacterial cultures were performed in 41 (64.1 %) patients. 17 were negative, 17 were positive and seven were positive after enrichment. Amongst others, the following bacteria were cultivated: Pasteurella, Staphylococci, Streptococci (Strep. equi ssp. zooepidemicus), E. coli, Enterococci, Pseudomonas, Borrelia, Actinobacilli, Acinetobacters, Enterobacters, Prevotella, methicillin resistant Staphylococcus aureus/pseudointermedius (MRSA, MRSP).

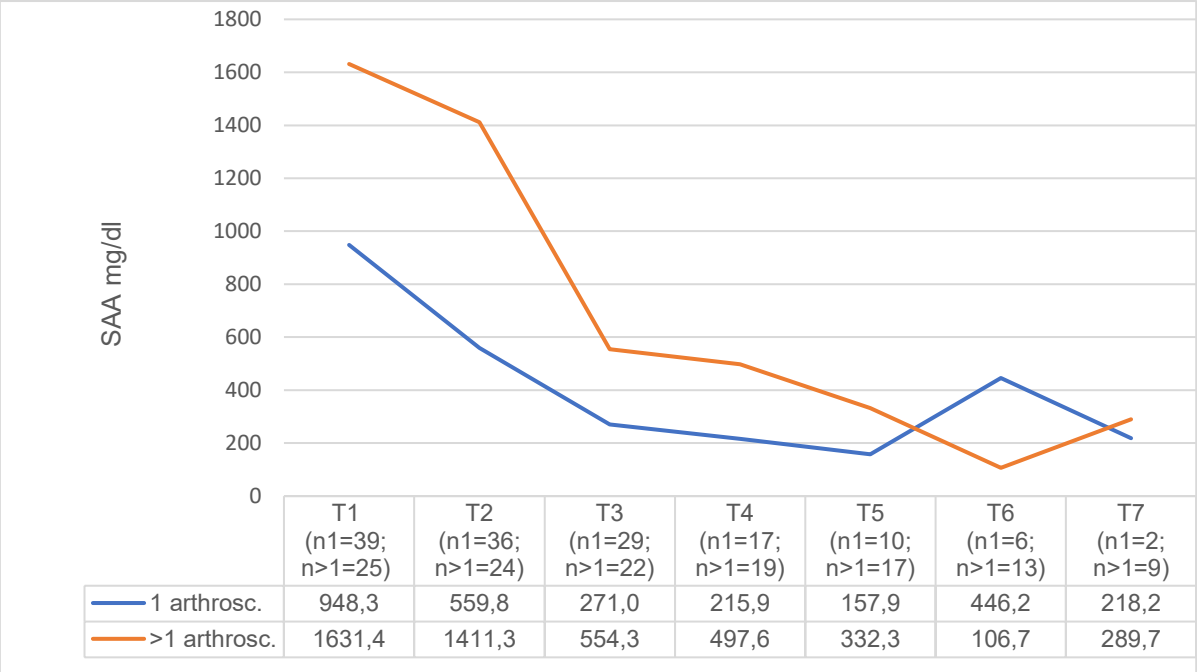
4.1.5 SAA

SAA was measured in regular intervals (48h) up to twelve times in some horses. For statistical analysis, SAA values were considered only until the 7th measurement timepoint post-surgery (T0 to T7), due to the small number of cases (n ≤ 8) at T8 and later.

Due to the retrospective study design, time points of SAA measurement varied between cases. In a total of 64 cases, the first SAA measurement was taken at the time of admission in 41 patients (T0 = 41 cases), whereas in the remaining cases (n=23) SAA was taken after surgery (usually within 12h after admission, further addressed as timepoint 1, T1). Detailed information on SAA levels at the different measurement time points are displayed in Appendix I: SAA in different points in time sorted in pre- and post-operative cases (Table and Figure).

For further analysis, our population was divided in horses that needed one lavage and horses that needed more than one lavage for clearance of septic synovitis. Figure 1 shows the course of SAA over time in these two groups. Results indicate that horses, needing one arthroscopy

(lavage) tend to have lower SAA levels after the first surgery (T1) than those with repeated surgery.



	T1 (n1=39; n>1=25)	T2 (n1=36; n>1=24)	T3 (n1=29; n>1=22)	T4 (n1=17; n>1=19)	T5 (n1=10; n>1=17)	T6 (n1=6; n>1=13)	T7 (n1=2; n>1=9)
1 arthrosc.	948,3	559,8	271,0	215,9	157,9	446,2	218,2
>1 arthrosc.	1631,4	1411,3	554,3	497,6	332,3	106,7	289,7

Figure 1. Median SAA values over time (T1 to T7 post OP) comparing horses receiving one vs more than one lavage

In general, a decline in SAA values could be observed in all horses after the second time point (T2) SAA values were determined (also see appendix I: SAA levels of all horses after the first arthroscopy).

4.1.6 Arthroscopy

Most of the horses, 39 (60.9 %) received only one arthroscopic lavage. In only eleven cases more than two lavages were needed (see also appendix I: frequency and percentages of arthroscopic lavages) (Figure 2).

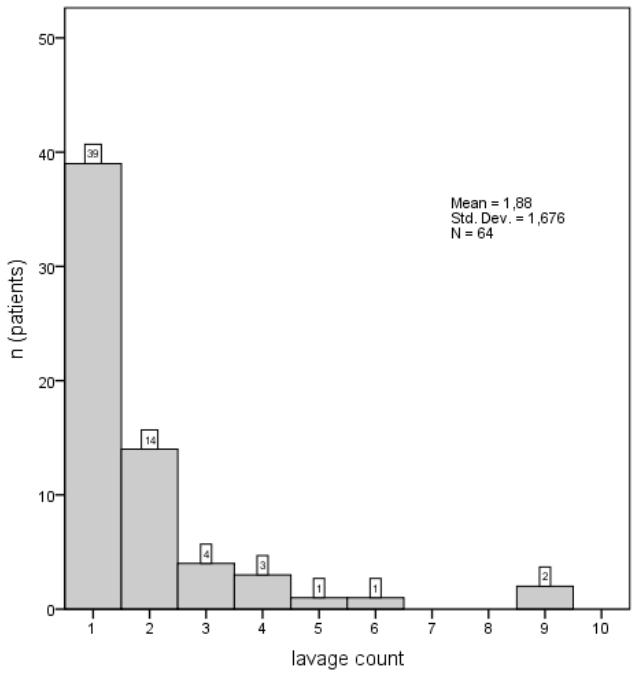


Figure 2. Frequency (n) of patients receiving lavage

4.1.7 Survival

From all horses (n=64), nine (14.1 %) had to be euthanized either due to poor prognosis and/or financial constraints. At the time of euthanasia according to the results of their synovial fluid analysis four still had septic synovitis (TNCC > 20 x 10^3 / µl, neutrophil granulocyte percentage > 80.0 % and increased total protein > 4 g/dL), two showed borderline (only one of the three parameters [TNCC, TP, Neutrophils increased], three negative results. Six horses (66.6 %) had chronic lesions (partly > one week), three had septic tenosynovitis (23.1 % of horses with tenosynovitis) and two septic bursitis (20 % of horses with bursitis within the study) either alone or additional to septic arthritis at admission. In contrast only four of 41 (9.8 %) that suffered from septic arthritis had to be euthanized. From the nine patients that had to be euthanized, in six cases bacterial examinations were performed and four of them were positive.

Euthanized horses tended to show higher median SAA levels after surgery (T1-T5) compared to horses that needed one or more lavages (Appendix I: Median SAA levels of Euthanized cases compared to Median SAA of pre- and post-surgery cases)

4.2 Analytical results

4.2.1 Duration of disease and changes in SAA

Median SAA levels of horses presented with injuries of less than 24h duration at the time of admission were significantly lower with a moderate effect (p = .039, r =.34) compared to horses with injuries older than 24h at the time of admission.

The median SAA values of patients with presurgical SAA measurements were lower than those with only postsurgical measurements (Appendix I: SAA in different points in time. sorted in pre- and post-operative cases (table and figure)). Median SAA values showed a significant increase (p = .018) from pre- to post-surgical (T1) measurements, with a small effect r = .26. SAA values decreased significantly after surgery between following time points: SAA T1 to T2 (p = .009, n=60), T2 to T3 (p <.001, n=51), T1 to T6 (p <.001, n=19) and T1 to T7 (p = .003, n=11) with small (T1-T2, r = -.24) to strong (T2-T3 r = -.51, T1-T6 r = -.58, T1-T7 r = -.63) effects.

Considering the different groups of horses (Figure 1), those that received one and those that received more than one arthroscopic lavage, SAA levels on timepoint one and time point two differ significantly (T1: p = .005; T2: p = .002) from each other.

4.2.2 Lavage

The relationship between SAA level on timepoint one after surgery and lavage frequency, revealed a weak to moderate connection r_s (n = 64) = .30, p = .016. The higher the SAA values after the first arthroscopic treatment, the higher was the frequency of performed arthroscopic lavages, in total.

4.2.3 Positive pressure test

In 33 cases data of positive pressure tests have been documented; 22 (66.7 %, 95% CI [50.6 % - 82.8%]) of these tests turned out positive revealing wound communication within synovial structures. The according confidence interval indicates that those tests turn out positive in more than 50 % of the times they are performed within the investigated population.

4.2.4 SAA levels and synovial fluid analysis

Based on the analysis of the results of synovial samples at admission, three categories could be defined: *nonspecific*, *borderline*, and *positive* cases. Cases that had increased TNCC's

(> 20 x 10^3 / µl), total protein (> 4 g/dL) and relative neutrophile granulocytes (>80 %) measured within their synovial sample were considered positive cases. Borderline samples were those that only had one of the three parameters increased, nonspecific cases were cases that had no increases in any of the parameters and/or were entitled as nonspecific by diagnosticians in the laboratory. In total, synovial analyses could be performed on 63 patient protocols as shown in
Table 2.

Table 2 *Key values and characteristics of SAA in mg/L and lg10 SAA considering 3 categories of synovial fluid analysis outcomes*

Synovia category	N	*M*	±*SD*	min – max	*Md*	IQR	mean rank
Nonspecific	5	789.8	893.9	130.1 - 2349.9	414.8	371.7 - 682.3	17.40
lg10		2.70	.46	2.11 - 3.37	2.62	2.57 - 2.83	
Borderline	21	1412.2	1047.8	9.2 - 3898.5	1265.2	428.6 - 1795.1	29.48
lg10		2.94	.58	.96 - 3.59	3.10	2.63 - 3.25	
Positive	37	5708.3	14031.8	77.7 - 57045.0	1443.6	850.0 - 3038.8	35.41
lg10		3.22	.59	1.89 - 4.76	3.16	2.93 - 3.48	
Total	63	3885.9	10934.2	9.2 - 57045.0	1348.5	664.7 - 2388.1	
		3.08	.60	.96 - 4.76	3.13	2.82 - 3.38	

According to the results of Kruskal-Wallis test SAA levels tended to increase considering the three categories of synovial fluid analysis (χ^2 (3) = 4.847, p = .089). Figure 3 illustrates the distribution of SAA levels (logarithmized y-axis) according to the three categories.

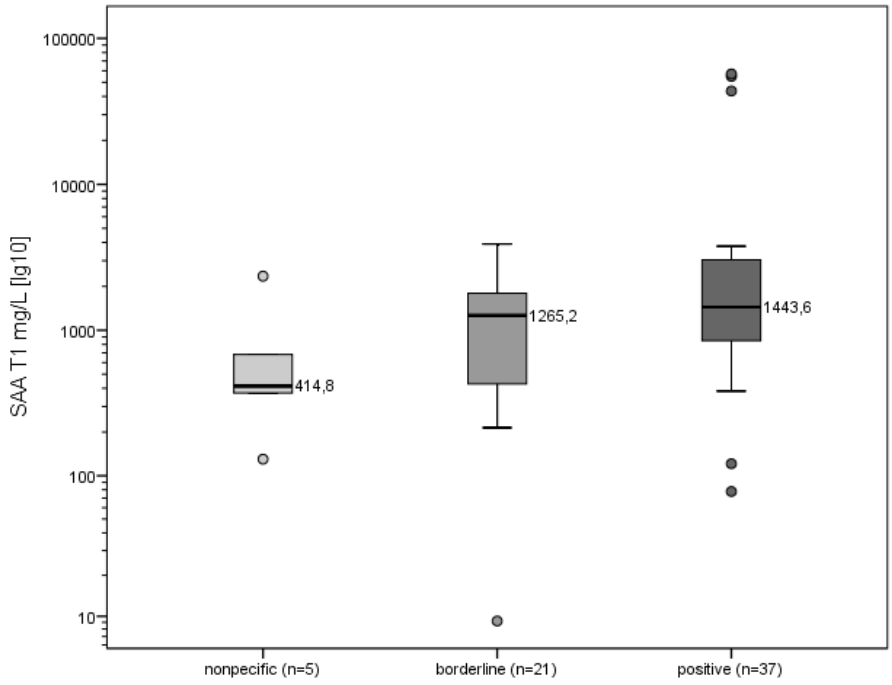


Figure 3. *Distribution of original SAA (T1) values (*Md*) with logarithmized y-axis*

4.2.5 SAA and bacterial examination outcome

No difference could be found in SAA levels, pre- and post-surgery of horses with and without a positive bacterial culture.

4.2.6 Model testing

A multilinear regression analysis was performed to see which of the parameters (results of synovial fluid analysis, wound age, age) influences SAA levels post OP, the most.

Results reveal that positive synovial fluid analysis outcome can be considered as a significant ($p < .05$) predictor for higher post-surgical SAA values compared to nonspecific and borderline synovial fluid outcomes, with a mild effect ($\beta = .268$). Age and wound age did not show a significant weight for prediction of post-surgical SAA level.

4.2.7 Discriminant analysis

As a supplementary approach to model testing, we performed a discriminant analysis. The aim was to see whether the patients could be assigned correctly into one of the synovial fluid analysis groups, if post-surgical SAA values, wound age, and individual age are known. These

values could be determined in 58 out of 64 cases. In these 58 cases that could be considered, 34 (58.6%) cases could be classified correctly. 20 out of 58 horses were falsely classified, whereas four positive horses were grouped wrongly as borderline ones (Table 3). Furthermore, with p = .188 only a tendency of right discrimination could be assumed by this analysis, but no significancy.

Table 3 Classification matrix indicating the quality of assignment (correct hits in the main diagonal)

Synovial fluid analysis		Predicted Group Membership			Total (100%)
		Nonpecific	Borderline	Positive	
Observed	Nonspecific	0	2 (40%)	3 (60%)	5
	Borderline	0	**5 (25%)**	15 (75%)	20
	Positive	0	4 (12.1%)	**29 (87.9%)**	33
	Total	0	11	47	58

5 Discussion

In this retrospective study, we collected data of (otherwise healthy) horses that underwent arthroscopy due to septic synovitis, to evaluate if systemic SAA is feasible as a monitoring tool for treatment response following surgery. Additionally, SAA values and their relation to results of synovial fluid analysis and duration of injury (e.g. wound age <24h or ≥24h) were evaluated.

With the results obtained in the current study we were able to verify our hypothesis that systemic SAA can be used as feasible monitoring tool in horses with septic synovitis. The median SAA level of all patients in the current study treated for septic synovitis decreased significantly from the second timepoint of evaluation after the first surgery. However, we observed higher median SAA values in horses receiving more than one arthroscopy (25 horses) after the first surgery than in patients with only one performed lavage. Median SAA levels after the first surgery were 948.3 mg/L in horses that received only one arthroscopic lavage (n=39) and 1631.4 mg/L in horses that received more than one arthroscopic lavages (n=25). This significant difference (p= .005) could also be observed at the second timepoint of measurement (T2) horses that needed more than one arthroscopy still had significantly higher median SAA levels (T2 >1 arthroscopy = 1411.3 mg/L, T2 1 arthroscopy = 559.8 mg/L, p = .002) than horses, that only needed one. These results suggest that systemic SAA levels decrease with – but stay higher in absence of – treatment success. This fact has also been reported previously in several studies. (Haltmayer et al. 2017, Jacobsen et al. 2005, Pepys et al. 1989) In these studies persistent high SAA levels post surgery, increasing SAA levels post surgery, respectively, could be related to post surgical complications (e.g. surgical site infections). Other possible reasons for persistently high SAA values may be – continuing inflammatory processes, persisting infection, a higher amount of tissue damage during surgery or due to excessive soft tissue trauma at the time of injury. (Haltmayer et al. 2017, Jacobsen 2009) We could observe an increase of median systemic SAA levels in horses after surgery. Pre surgical measurements were only available from a part of the study population (n=41) due to restriction of analyzing equipment during emergency services. From these 41 horses evaluated in our study pre-surgery, 34 had SAA levels above the cut off value of 10 mg/L at admission (*Md* = 479.6 mg/L), whereas 63 (out of 64) horses had SAA levels higher 10 mg/L after surgery (*Md* = 1349.9 mg/L). The reason for high SAA values post-surgery may be local inflammation due to surgical trauma in combination with tissue damage from the original injury, as described in previous studies. Therefore, SAA rises post surgery in relation to the amount of tissue trauma – the more tissue is involved the higher the SAA. On the other hand high

SAA levels post surgery may also indicate the need for a more aggressive treatment in order to resolve the septic synovitis. (Jacobsen et al. 2006, Jacobsen 2009, Pepys et al. 1989, Pollock et al. 2005)
Arthroscopic lavage and through and through lavage per se, don't lead to SAA level increases, but to increases in TNCC, and total protein post surgery. (Sanchez-Teran et al. 2016 (a), Sanchez-Teran et al. 2016 (b))

General anesthesia may also have an influence on postsurgical SAA values, but contradictory results have been published in the past. One study showed no increase in SAA values in horses receiving only general anesthesia (n=2). (Pepys et al. 1989) Another study did show significant differences in SAA levels in horses under general anesthesia with and without performed surgery but median SAA values of 520.7 mg/L after general anesthesia alone (n=7) (without surgical intervention) indicated an influence of anesthesia on SAA levels. (Stowasser-Raschbauer et al. 2013)

Depending on the duration of injury prior to admission reported by the horses' owners, median SAA levels were 220.4 mg/L in horses with injuries younger than 24h (n=13) and 724.6 mg/L in horses affected for more than 24h (n=24) prior to admission. In contrast, no difference between duration of injury and SAA values after surgery could be observed. In 37 horses wound age and pre-surgical SAA values were evaluated and tested for differences. Our data showed significantly lower SAA levels in horses with injuries of < 24h duration and higher SAA measurements in horses with problems lasting > 24h. These results matched results found in previous studies. (Haltmayer et al. 2017, Jacobsen et al. 2006, Müller et al. 2021, Nunokawa et al. 1993) Since systemic SAA peaks only after 48h, horses with injuries < 24h may have been evaluated too early for SAA to reach its final concentration. These would also explain the overall lower SAA values in horses admitted < 24h after the onset of lameness, after injury respectively.

This characteristic pattern of SAA (peak at 48h, followed by decrease) may also explain the overall decrease in SAA over time. Once the initial acute phase reaction stimulated by the injury, infection, surgery and general anesthesia subsides, SAA levels decrease to some extent, but still stay higher in patients with lack of treatment success (Fig.1, Appendix I: Median SAA of Euthanized cases compared to Median SAA of pre- and post-surgery cases).

Up to date synovial fluid analysis is the gold standard for diagnosis of synovial sepsis. Therefore, we analyzed the correlation between SAA levels and results of synovial fluid

analysis. We could observe a tendency, that positive (=septic) synovial fluid analysis outcomes (n=37) lead to higher median SAA values than borderline (n=21) or nonspecific (n=5) synovial fluid outcomes. Therefore, using a multiple linear regression model we analyzed the influence of patient age, wound age, and results of synovial fluid analysis on post-operative systemic SAA values. We were able to show that a positive synovial fluid outcome influences SAA levels after surgery the most. Wound age and patients' age did not have any explanatory value on SAA levels post-surgery in the current study.

This suggests that repeated measurements of systemic SAA post-surgery is feasible as a monitoring tool for treatment success regardless of duration of injury at the time of admission.

Moreover, systemic SAA values seem to reflect the current state of a synovial structure quite accurately and therefore provide an easy and inexpensive additional tool for monitoring treatment success after surgery due to septic synovitis reducing the need for repeated synoviocentesis. Synoviocentesis as monitoring tool can be difficult in horses due to several reasons. Drainage of wounds, fibrinous flocculation or pannus can prevent successful sample collection. (Bertone and Cohen 2011, Haltmayer et al. 2017) Also, repeated administration of antimicrobials may cause increased synovial fluid parameters, which than could be confused with synovial sepsis. (Dykgraaf et al. 2007, Sanchez Teran, Rubio-Martinez, Villarino, Sanz 2012) Systemic SAA on the contrary seems to remain uninfluenced and can therefore not only provide easy and inexpensive, but also unbiased information considering synovial status despite local antimicrobial injection. (Sanchez Teran, Rubio-Martinez, Villarino 2012) Furthermore, repeated synoviocentesis bears a risk of reinfection of the actually cured synovial sepsis, which could be prevented with systemic SAA measurements. (Adams et al. 2010, Wahl et al. 2012)

Overall, we found that systemic SAA decreased over the course of treatment after surgery. However, we could observe a rise in SAA between timepoint T6 and T7 which was caused by one horse whose SAA value increased for no obvious (clinically visible) reasons and decreased again on T8. One of the two horses with a total of twelve SAA measurements, increased again as high as 3056.2 mg/L at the 12^{th} time and was euthanized due to pododermatitis purulenta profunda with involvement of bursa podotrochlearis. Two horses received seven SAA values although only one lavage has been performed. The reason for this was non improving clinical presentation in one horse, and wound dehiscence in the other one

representing the need of the clinicians in charge to check potential ongoing inflammatory processes.

Two horses received up to nine lavages. One of these two was euthanized due to osteomyelitis and poor prognosis, the other was recovering from the septic synovitis of the elbow joint.

From nine euthanized horses, five horses received more than one lavage, three had septic tenosynovitis (23,1% of all horses with septic tenosynovitis) and two septic bursitis (20% of all horses with septic bursitis) in combination with or without a septic joint, suggesting potential higher risk of non-survival when bursae or tendon sheaths are involved compared to septic arthritis (9,8% of all horses with septic arthritis). Similar survival rates for septic tenosynovitis and septic arthritis were reported by other authors. In our study horses with septic bursitis had a slightly better survival rate (80%) compared to previous studies (67%) (Frees et al. 2002, Gibson et al. 1989, Honnas et al. 1991, Post et al. 2003, Schneider et al. 1992)

Considering the course of median SAA levels of non-survivors, a decrease of SAA could only be observed starting at T2 in contrast to survivors, where SAA started to decrease after the first surgery (T1). Moreover, the median SAA values are higher until T5, than those of all horses together (surviving and non-surviving horses) (Appendix I: Median SAA of Euthanized cases compared to Median SAA of pre- and post-surgery cases). Therefore, high SAA levels after the first surgery could potentially indicate complications and a prolonged or unsuccessful recovery. Exceptional high SAA levels after the first surgery (Md: 1860.1 mg/L in euthanized, compared to 1348.5 mg/dL in survival horses) could aid in earlier detection of postoperative complications (i.e. persisting infection), lead to more aggressive treatment approach and potentially avoid fatal consequences for the patient.

Due to the different etiology and concomitant systemic diseases (polyarthritis, septicemia) in foals and different reference values in healthy foals, only horses > 1 year of age were included in this study. (Haltmayer et al. 2017, Jacobsen 2009) Furthermore, all horses that could have increased SAA levels for other reasons except septic synovitis – like gastrointestinal, respiratory, or other inflammatory diseases – were excluded (Nunokawa et al. 1993, Pihl et al. 2015).

Although Serum Amyloid A seems to present a good tool for monitoring treatment success in septic synovitis, its' specificity is limited due to its' nonspecific systemic reaction in every inflammatory process, as all acute phase proteins. Diseases of gastrointestinal, respiratory or other, systemic, origin lead to SAA values similar to SAA levels reached by each horse due to

septic synovitis, which is the reason that SAA measurements can only be used as additional tool and can’t replace synovial fluid analysis. (Long and Nolen-Walston 2020, Witkowska-Piłaszewicz et al. 2019)

Limitations of this study are the retrospective design and the different decisions of the clinicians in charge. So that the comparability between cases was not as exact as prospective study designs may have provided. A further limitation is the missing control group, that was not available in the clinical setting.

6 Conclusion

The present study suggests that Serum amyloid A is a feasible monitoring tool for treatment success in horses recovering from septic synovitis. After the prior increase of median SAA levels in horses that only needed one arthroscopic lavage, median SAA levels decreased over time until <10 mg/L. In horses that needed more aggressive treatment as a second or third arthroscopic lavage, SAA values increased to higher levels and stayed higher for a longer time, then those sufficiently treated with one arthroscopic lavage. Persistently high SAA (over more than 3 timepoints) values in our study lead to euthanasia in six cases and therefore potentially indicate missing treatment success. Furthermore, (considering also clinical examinations) SAA levels seem to reflect synovial fluid condition, at least in positive results, so that SAA can be seen as helpful additional diagnostic tool to synovial fluid analysis.

7 Preview

Introduction: Septic synovitis in horses - if not treated promptly - has serious consequences for the patients' sportive use, and its survival in general. The present study deals with the inflammatory marker serum amyloid A as a financially beneficial monitoring tool for treatment success in this serious condition.

Materials and Methods: Data of 64 hospitalized equine patients, presented to the Equine Clinic of Vetmeduni Vienna with septic synovitis, over the period of 5 years (2015 - 2020), were collected: Duration of symptoms (<24h, ≥24h), affected synovial structure, synovial fluid analyses, SAA values, and number of arthroscopies needed, were recorded from each patient. For statistical analysis a Wilcoxon matched paired samples test was used to compare SAA levels at different time points (T0-T8), Spearman rank correlation and a multiple linear regression were used to compare SAA values considering the number of arthroscopic lavages needed by each horse, and to analyze a possible correlation between SAA levels and results of synovial fluid analysis.

Results: Median SAA values decreased significantly (p = .009), after the first surgery. Patients that needed more than one arthroscopy had significantly higher (p = .005) postsurgical median SAA values (1631.4 mg/L) compared to those that only needed one (948.3 mg/L) lavage. Horses that were euthanized had the highest median SAA values at the second timepoint of SAA measurement after surgery (T1: 1860.1 mg/L, T2: 2475.8 mg/L). Multiple linear regression defined positive synovial fluid analysis results as significantly best predictor for increased postsurgical SAA levels (p = .042), compared to age or wound age.

Conclusion: The present study suggests that SAA levels seem to reflect results of synovial fluid analysis relatively accurate and are therefore a good tool for monitoring treatment success in horses suffering from septic synovitis

German preview:

Einleitung: Septische Synovitiden beim Pferd haben schwerwiegende Folgen für den sportlichen Einsatz der Patienten und ihr Überleben, wenn sie nicht rechtzeitig behandelt werden. Die vorliegende Studie befasst sich mit dem Entzündungsmarker Serum-Amyloid A als finanziell günstiges Überwachungsinstrument, für den Behandlungserfolg bei dieser Erkrankung.

Materialien und Methoden: Es wurden Daten von 64 hospitalisierten Pferdepatienten gesammelt, die über einen Zeitraum von 5 Jahren (2015 - 2020) an der Pferdeklinik der Vetmeduni Vienna mit septischer Synovitis vorgestellt wurden. Es wurden, die Dauer der Symptome (<24h, ≥24h), die betroffene synoviale Struktur, Synovia-Analysen, SAA-Werte und Anzahl der notwendigen Arthroskopien, von jedem Patienten erhoben. Verschiedene statistische Methoden (Mann-Whitney-U-Test, Wilcoxon-Test für gepaarte Stichproben, Kruskal-Wallis-Test, Spearman-Rangkorrelation, multiple lineare Regression) wurden angewandt, um Veränderungen im Verlauf der SAA-Werte festzustellen, die SAA-Werte post-OP unter Berücksichtigung der Anzahl der Arthroskopien zu vergleichen und herauszufinden, ob die ermittelten SAA-Werte, die Ergebnisse der Synovia-Analyse mit einer vorgegebenen Wahrscheinlichkeit von p = .05 ausreichend widerspiegeln.

Ergebnisse: Die SAA-Werte verringerten sich signifikant unter Berücksichtigung des Medians in allen Fällen, nach der ersten Operation (p = .009). Patienten, die mehr als eine arthroskopische Spülung benötigten, hatten signifikant höhere (p = .005) postoperative mediane SAA-Werte (1631,4 mg/L) im Vergleich zu denen, die nur eine Operation benötigten (948,3 mg/L). Pferde, die euthanasiert wurden, hatten die höchsten medianen SAA-Werte zum ersten und zum zweiten Zeitpunkt der SAA-Messung nach der Operation (T1: 1860,1 mg/L, T2: 2475,8 mg/L). Mithilfe der Multiplen linearen Regression, ließ sich errechnen, dass in unserer Studie, der größte Einfluss auf postoperative SAA Werte, durch positive Synovialbefunde gegeben war (p = .042). Gar kein Einfluss erzielten Alter und Wundalter.

Schlussfolgerung: Die vorliegende Studie legt nahe, dass die SAA-Werte, die Ergebnisse der Synovia-Analyse relativ genau widerspiegeln und daher ein gutes Instrument zur Überwachung des Behandlungserfolgs bei Pferden mit septischen synovialen Strukturen sind.

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9 Tables

10 Figures

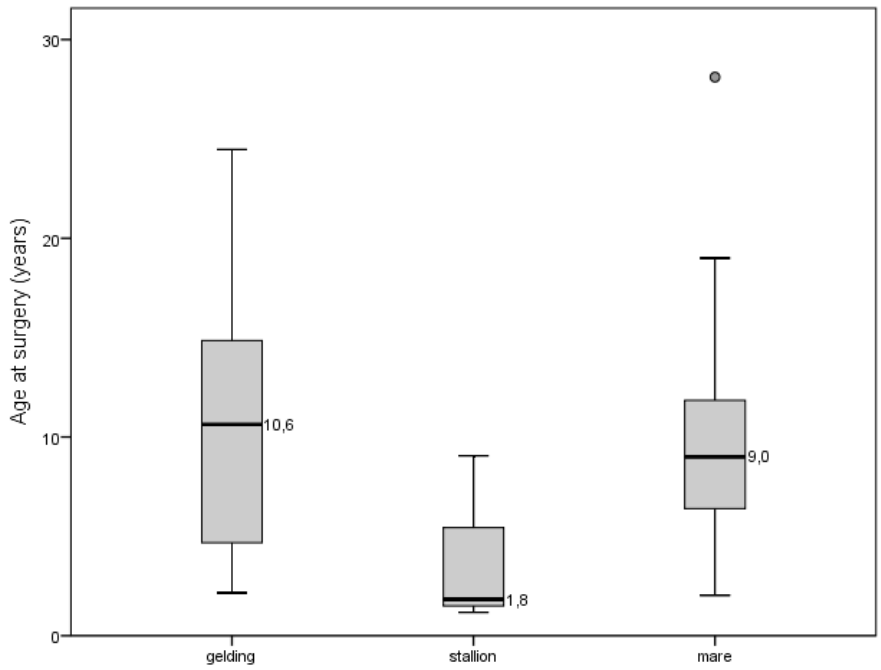
11 Index of abbreviations

Abbreviation	Description
CT	Computed tomography
IQR	Interquartile range
IVRLP	Intraveneous regional limb perfusion
M	Mean
Max	Maximum
Md	Median
Min	Minimum
MRI	Magnet resonance imaging
SAA	Serum amyloid A
SD	Standard deviation
T1,2,3,..7	Timepoint 1,2,3..7
TIS	Tierspitalsinformationssystem

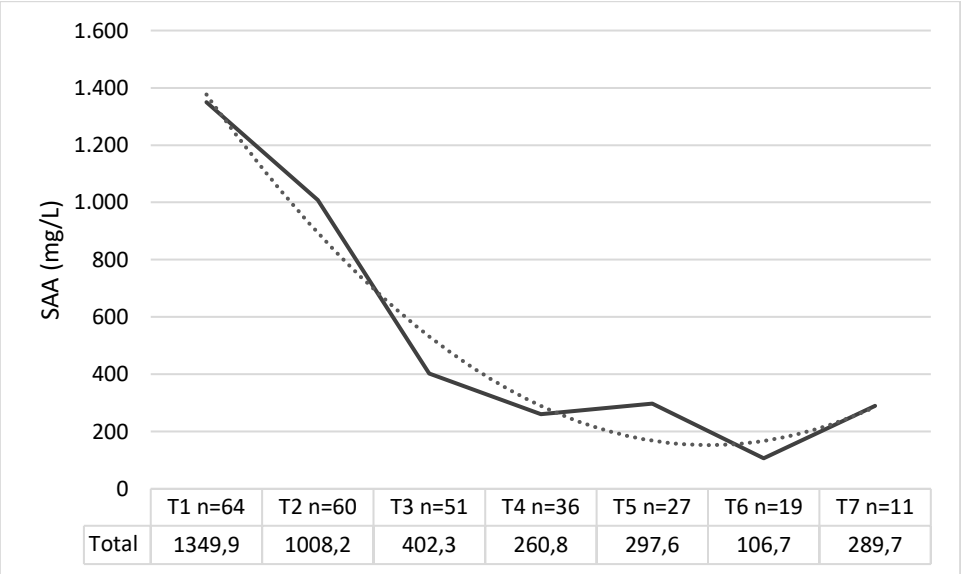
12 Appendix

Appendix I: Further Figures and Tables

Age and sex distribution, Boxplot



SAA levels of all horses after the first arthroscopy



	T1 n=64	T2 n=60	T3 n=51	T4 n=36	T5 n=27	T6 n=19	T7 n=11
Total	1349,9	1008,2	402,3	260,8	297,6	106,7	289,7

SAA in different points in time, sorted in pre- and post-operative cases (Table and figure)

Table: Key values of SAA levels received in different time points (pre = before. T1. T2.…= after surgery)

time of SAA measure		Pre	T1	T2	T3	T4	T5	T6	T7
Pre	N	41	41	37	31	23	20	15	8
	M	2305.9	2834.3	2189.9	905.5	747.0	454.3	397.5	315.6
	SD	8655.5	8392.7	6469.2	1136.5	968.2	703.0	539.5	298.9
	Min	1.0	9.2	.0	.0	.0	.0	.0	4.4
	Max	55673.0	54853.0	39918.0	4965.6	4462.6	3010.8	1680.3	856.5
	Md	479.6	1351.3	989.4	477.5	447.6	299.8	26.2	356.7
Post	N		23	23	20	13	7	4	3
	M		5733.6	5781.0	2555.1	493.7	420.4	690.2	163.7
	SD		14256.6	15226.8	8829.9	800.9	534.5	1042.8	283.2
	Min		130.1	5.2	.0	.0	.0	3.4	.0
	Max		57045.0	60978.0	39899.0	2701.1	1348.2	2239.3	490.7
	Md		1315.6	1153.5	322.4	193.2	172.5	259.1	0.5
Total	N	41	64	60	51	36	27	19	11
	M	2305.9	3876.3	3566.5	1552.4	655.5	445.5	459.2	274.2
	SD	8655.5	10847.3	10728.0	5573.5	907.9	653.7	650.1	289.1
	Min	1.0	9.2	.0	.0	.0	.0	.0	.0
	Max	55673.0	57045.0	60978.0	39899.0	4462.6	3010.8	2239.3	856.5
	Md	479.6	1349.9	1008.2	402.3	260.8	297.6	106.7	289.7
	IQR PR 25	38.8 - 1364.7	664.7 - 2445.8	253.1 - 1737.4	55.2 - 1118.6	109.8 - 970.0	19.7 - 516.5	8.8 - 830.2	8.6 - 460.3

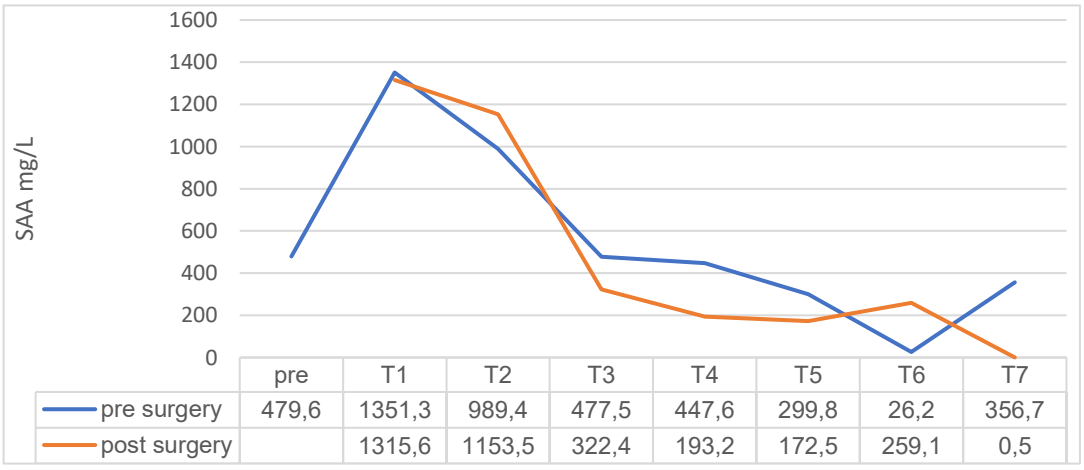


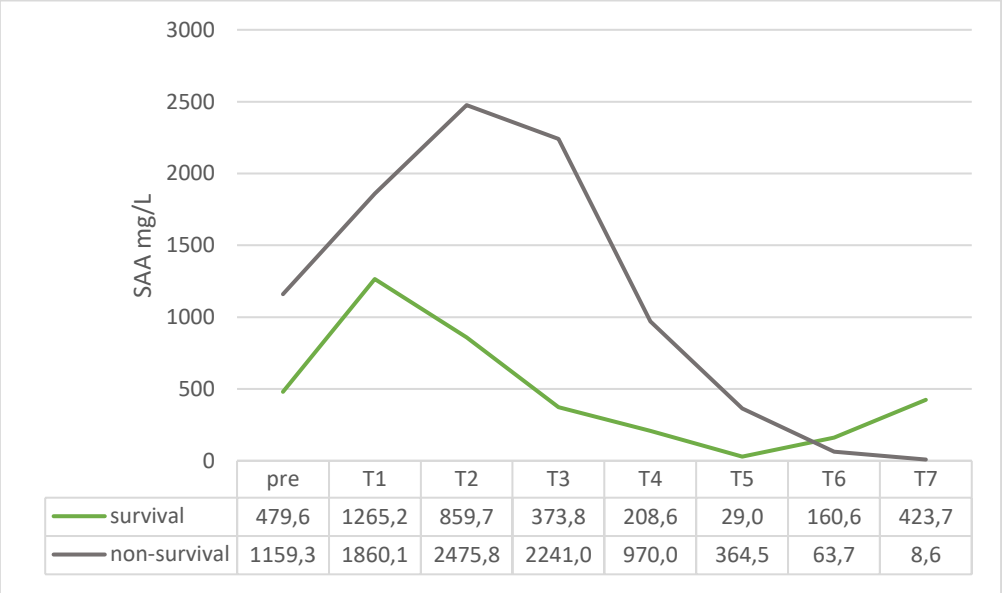
Figure. Median SAA values until the 8th time (T0 pre OP, T1 post OP, T2 second point of time after surgery, …) comparing horses with pre-surgery values vs those without this information

Frequnecy and percentages of received lavages

Table: *Frequency and percentage of patients receiving lavage*

Lavage count	n (patients)	Percent	Cumulative Percent
1	39	60,9	60,9
2	14	21,9	82,8
3	4	6,3	89,1
4	3	4,7	93,8
5	1	1,6	95,4
6	1	1,6	97
9	2	3,1	100
Total	64	100,0	

Median SAA levels of Euthanized cases compared to Median SAA of all cases



Appendix II: Model testing – multilinear regression

Coefficients for the predictors of criterion SAA post-surgery (T1) (n=58)

Model step	Predictor	Unstand. Coeff.		Stand. Coeff.	*T*	*p*-value	95.0% CI B	
		B	*SE*	B			LB	UB
1	(Constant)	2.805	.296		9.472	<.001	2.211	3.399
	Age at OP	-.013	.015	-.110	-.835	.407	-.043	.018
	Wound age	-.022	.164	-.018	-.137	.892	-.352	.307
	Synovia D2	.247	.305	.193	.808	.423	-.365	.859
	Synovia D3	.546	.299	.446	1.830	.073	-.052	1.145
2	(Constant)	2.802	.293		9.579	<.001	2.216	3.389
	Age at OP	-.013	.015	-.112	-.856	.396	-.043	.017
	Synovia D2	.241	.300	.189	.805	.425	-.360	.842
	Synovia D3	.538	.289	.438	1.861	.068	-.042	1.117
3	(Constant)	2.991	.174		17.178	<.001	2.642	3.340
	Age at OP	-.012	.015	-.107	-.827	.412	-.043	.018
	Synovia D3	.344	.159	.281	2.159	.035	.025	.663
4	(Constant)	2.886	.119		24.238	<.001	2.648	3.125
	Synovia D3	.329	.158	.268	2.084	.042*	.013	.645

*$p \leq .05$

Discriminant analysis

Test value Wilks-Lambda indicating the discriminant functions for significance

Test of Function(s)	Wilks' Lambda	χ^2 (df)	*p*-value
1 through 2	.850	8.745 (6)	.188
2	.998	.106 (2)	.948

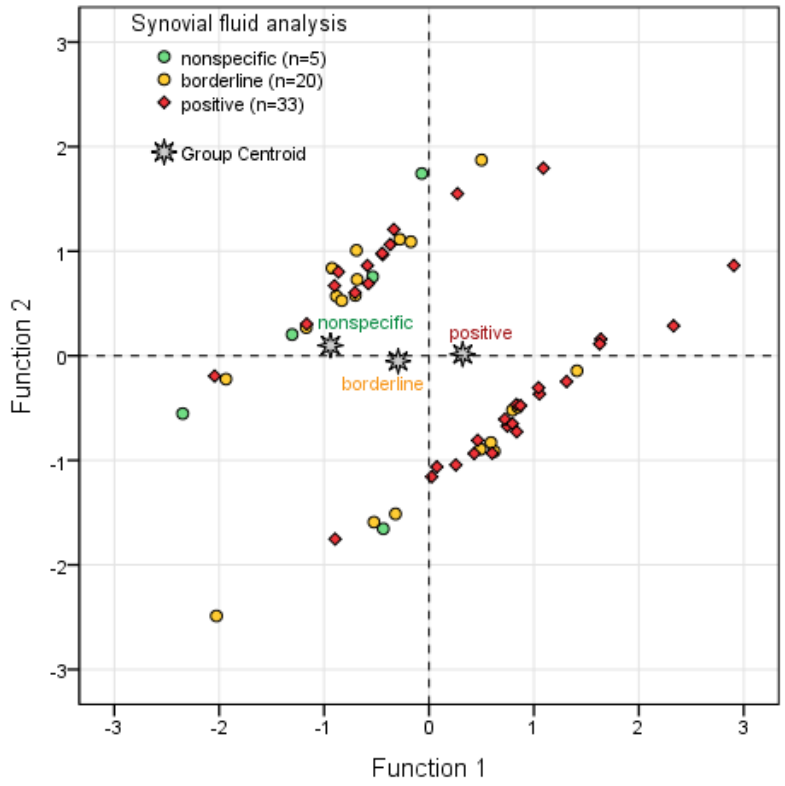
Structural matrix with factor loadings and standardized canonical discriminant function coefficients

Parameter Function	Structural matrix		Standardized Canonical Discriminant Function Coefficients	
	1	2	1	2
SAA T1 (log)	**.715***	.499	.770	.520
Age at OP (in years)	**.292***	.254	.335	.377
Wound age (<24h / ≥24h)	.595	**-.796***	.590	-.810

*Largest absolute correlation between each variable and any discriminant function Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions.

Coordinates of group centroids (unstandardized canonical discriminant functions evaluated at group means)

Group	Function (group centroids)	
	1	2
nonspecific	-.939	.099
Borderline	-.295	-.051
Positive	.321	.016



Bivariate scatterplot showing the position of each case using canonical discriminant function and group centroids of synovial fluid outcome (n=58)

13 Acknowledgements

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