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Comparison of Synovial Fluid Composition in Distended and Normal Digital Flexor Tendon Sheath of Horses (A Pilot Study)

Zahra Noori, Samaneh Ghasemi, Ali Mirshahi, Mehrdad Mohri* Ahmadreza Mohamadnia*

Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

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ABSTRACT

Diagnosis of tenosynovitis is usually confirmed on the basis of synovial fluid analysis (such as cytological and biochemical examinations). This study was designed to examine biochemical (serum amyloid A (SAA), haptoglobin (Hp) and D-dimer) and cytological (total nucleated cell count (TNCC), neutrophil, lymphocyte, and monocyte count) characteristics of serum and synovial fluid of the equine digital flexor tendon sheath (DFTS). Synovial fluid samples were aseptically collected in EDTA from 43 limbs in 20 horses with (study group) and 8 limbs in 8 horses without (control group) distention of DFTS and serum samples were collected from each horse. Lymphocyte, monocyte, neutrophil, and TNCC in the synovial fluid were statistically higher in the distended sheath that shows an inflammatory nature of the distention, however, concentrations of D-dimer was lower in serum (0.1, 0.10-3.80) and (0.2, 0.10-0.20) than synovial fluid (19.2, 17.78-20.00) and (20, 19.90-20.00)) in both groups. The serum SAA concentrations of the control group (1.7, 0.10-2.16) were significantly higher than the study group (1, 0.86-1.05) and the concentration of SAA in serum was higher than synovial fluid (0.89, 0.86-0.98) in the control group. Results of this current study show that a cytological evaluation of the synovial fluid is more valuable than biochemical findings in the diagnosis of the inflammatory nature of this condition.

Introduction

Digital flexor tendon sheath (DFTS) is an important synovial structure of the equine distal limb, Synovial fluid of joints, tendon sheaths, and bursa normally functions as a biologic lubricant and a biochemical pool through which nutrients and regulatory cytokines traverse.[1,2] The volume of fluid within the DFTS of horses varied minimally, with a mean of 2.11 ml.[3] In traumatic limb injuries, the DFTS is the most affected structure,[4] and consider as a cause of distension in tenosynovitis.[5]

Distension of DFTS can be diagnosed by palpation of the digital sheath, flexion test of the distal limb joints, local analgesic techniques,[6,7] contrast radiography[5], ultrasonographic examination,[5-7] magnetic resonance imaging (MRI)[7], endoscopy,[5-9] computed tomography (CT),[5,6] and digital sheath synovial fluid (SF) evaluation.[7] Measurement of total protein (TP) concentration and cytological examination of the SF by

* Correspondence to: Ahmadreza Mohamadnia and Mehrdad Mohri, Department of Clinical Sciences, Mashhad, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran. E-mail: mohamadnia@um.ac.ir; mohri@um.ac.ir

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tenovaginocentesis, along with the examination of clinical signs (lameness, synovial effusion, heat, etc.), can help to diagnosis and an appropriate treatment plan for injuries involving the DFTS.[8,10]

Flexor tendon sheath fluid (especially DFTS in horses) has a character (total leukocyte count, viscosity, and protein concentration) similar to SF of joints and has specific functions such as soft tissue lubrication and nutrition of avascular tendon tissue.[3,8,11] Total nucleated cell count (TNCC) provides rapid information for SF classification as a low or high cellularity effusion, which is indicative of different etiologies including inflammation,[12] and joint infection.[13] Acute Phase Response (APR) is a rapid, nonspecific response that may be related to any form of tissue damage including infection, trauma, neoplasia inflammation, stress, immunological disorders, or any other disturbances of homeostasis,[14-16] In response to inflammation, the plasma concentrations of negative acute proteins phase (APPs) decrease, and positive APPs increase. Positive APPs include haptoglobin (Hp), C reactive protein, ceruloplasmin, fibrinogen, and serum amyloid A (SAA). The positive APPs are further classified as major, moderate, and minor APPs.[14] Serum amyloid A is the major and most sensitive APP in horses and increases rapidly after the injury, infection, and inflammation, and reach to the highest amount in plasma within 48 hours.[14,15] In healthy horses, SAA is present at very low or trace levels, but it increases rapidly (100–1000 folds) in response to acute inflammation. High SAA concentrations have been reported in aseptic inflammation, surgical trauma and bacterial and viral infections.[15] also, the short half-life of SAA causes a rapid decrease in its concentration is observed in response to treatment, and resolution of the disease process,[14,15] and when inflammation resolves, SAA concentration decrease within 12 hours.[16] Haptoglobin is known as a marker of inflammation in serum and SF. It is a moderate acute phase protein in horses and remains high for a longer period.[12,17] D-Dimer is a specific indicator for general and local fibrinolysis activity, and a useful and sensitive test for evaluating coagulation and fibrinolysis increases in humans, dogs, and horses. D-dimer increases in inflammation, infection, thrombosis, or neoplastic disorders. Increased concentration of D-dimer in SF depends on the fibrinolytic activity of involving joints.[18] Inflamed synovium can produce a large amount of fibrin that can increase the D-dimer concentration in SF and serum.[19] D-Dimer is a useful marker of fibrinolysis in horses after endurance competition, in those with acute laminitis, colic, osteochondritis dissecans (OCD), foals with septic joint, and in septic foals.[20-22] This current study was designed for the evaluation of inflammatory biomarkers (TNCC, leukocyte differential counts, SAA, Hp) and hemostatic biomarker (D-Dimer) in the SF of distended DFTS in comparison to serum to evaluate its inflammatory or infectious nature.

Materials and Methods

Horses

Twenty-eight adult horses of different breeds, aged 2 to 12 years were enrolled in the study. Horses were clinically examined for general health status and lameness, didn't receive any medications recently, and without any obvious signs of infectious diseases. Horses and limbs are divided into two (control and study) groups. Eight horses (8 limbs) were assigned in control group. The main criteria for selection of the horses in control group were lack of any distention in DFTS, inflammatory changes in CBC and lack of any clinical manifestation of lameness in clinical examination. Twenty horses (43 limbs) with distension of DFTS (effusion of DFTS, Tendon thickening, and pain during the tendon sheath palpation) and different stages of clinical lameness with inflammatory changes in CBC were assigned in the study group.

Synovial Fluid Collection

Horses were examined for DFTS-related problems. Synovial effusion (SE) was ranked by a semi-quantitative five-point scale method. Zero for without palpable effusion, 1 for slight effusion in the upper sac of the tendon sheath, two for slight effusion at the top and bottom of the tendon sheath, three for moderate effusion and distention in the top and bottom of the tendon sheath and, four for a pronounced dilatation of the tendon sheath.[8] The jugular blood and SF from the DFTS were taken from all horses. Synovial fluid was taken through the palmar axial sesamoidean approach from DFTS in EDTA tube. Difficulty in SF sampling (DS) was ranked between 0 to 4 on a five-point scale. Score 0: fluid is easily aspirated; 1: the needle is replaced less than 2 times before aspiration, 2: fluid is taken with difficulty and the needle is replaced for more than 3 times, 3: the proximolateral approach is used for the synthesis, and 4: the proximolateral approach is used for the synthesis; fluid is taken with difficulty.[8]

Biochemical and Cytological Evaluation of SF of DFTS and Serum

Cytological evaluation (TNCC and differential count) was performed on all SF samples before freezing and blood and SF samples were centrifuged at 1800 g for 15 minutes and serum was frozen at -80° C until doing further assessments. Serum amyloid A and Hp were measured using ELISA kits (Bioassay Technology Laboratory, China) and D-Dimer was measured by immunoturbidimetric assay with NycoCardTM D-Dimer (Nycomed Pharma, Switzerland).

Statistical Analysis

The results were analyzed by using the statistical package SPSS version 24.0. The normality of data was assessed by Shapiro-Wilk and Kolmogorov-Smirnov statistical tests (level of significance $p < 0.05$). An independent t-test and Mann-Whitney test were used to compare the concentration of parameters between the horses in two groups. A paired t and Wilcoxson test was used to analyze the concentrations of the parameters between serum and SF of horses and serum of any horse has been compared with the maximum amount of SF. Data of SE and DS scores were analyzed by analysis of variance (ANOVA) and the Kruskal-Wallis test. In statistical analysis, groups with numbers less than 5 in DS and SE scores were deleted.

Results

Table 1 presents the results of analyzing the SF of DFTS and serum of horses participating in this study. The evaluation of SF indicated that the study group showed a significant increase ($p < 0.05$) in the amounts of TNCC, lymphocyte, monocyte, and neutrophil compared to the control group. Comparison of the serum in horses of study and control groups showed a significant difference ($p < 0.05$) in concentrations of SAA. Comparison of serum and SF in each horse showed that concentrations of synovial fluid D-dimer in study and control groups were significantly higher than serum and concentrations of serum SAA in the control group were significantly higher than synovial fluid ($p < 0.05$) (Table 1). Synovial effusion score: the highest number of horses was in Score 2 (n = 11) and the rest of scores included 3 (n = 9), 0 (n = 8), 1 (n = 6) and 4 (n = 6) (numbers were equal in 1 and 4). Statistical analysis of groups in SE score indicated that scores 0 and 3 were significantly different ($p < 0.05$) in terms of lymphocyte count, neutrophil count, and TNCC count (Table 2).

Table 1. Median and Percentiles (Q1, Q3) or Median (minimum–maximum) of biochemistry and cytology variables in serum and SF of control and study groups.

	Groups	Serum Median (Q_1, Q_3) No	SF Median (Q_1, Q_3) or (Min- Max) No
D-Dimer mg/l	Control*	0.1 (0.10, 3.80) 8	19.2 (17.78, 20.00) 8
	Study*	0.2 (0.10, 0.20) 8	20 (19.90, 20.00) 41
	p value	0.641	0.109
SAA µg/ml	Control*	1.7 (0.10, 2.16) 8	0.89 (0.86, 0.98) 8
	Study	1 (0.86, 1.05) 28	0.96 (0.87, 1.07) 43
	p value	0.013**	0.233
Hp µg/ml	Control	38.85 (37.21, 43.79) 8	38.85 (35.25, 40.09) 8
	Study	37.82 (35.66, 39.26) 28	36.38 (34.74, 39.26) 43
	p value	0.083	0.317
TNCC* (cells/µl)	Control		0.00 (0.00- 100.00) 8
	Study		400.00 (0.00- 8050.00) 30
	p value		0.00**
Lymphocytes* (cells/µl)	Control		84.00 (45.00- 95.00) 7
	Study		329.00 (67.00- 6601.0) 29
	p value		0.00**
Monocytes* (cells/µl)	Control		6.00 (1.00- 14.00) 7
	Study		23.00 (0.00- 1224.00) 29
	p value		0.04**
Neutrophils* (cells/µl)	Control		0.00 (0.00- 10.00) 7
	Study		20.00 (0.00- 644.00) 29
	p value		0.00**

**Significantly different between two groups ($p < 0.05$).
* Significantly different between SF vs serum in each group ($p < 0.05$).

The approach was not changed in the horse sampling; hence, no horse was in score 4 in the DS scoring. In most cases, sampling was easily performed; hence, the highest number of horses was in score 0 (n = 23); and other scores included 1 (n = 13), 2 (n = 2), and 3 (n = 2) respectively. In the DS scoring, there was a statistically significant difference ($p < 0.05$) between scores 0 and 1 in amounts of monocytes, but other variables were not statistically different in SE and DS scoring (Table 3).

Discussion

Distension of DFTS is common and is not necessarily associated with lameness,[5] especially in hind limbs.[8]

Although few studies have specifically measured some criteria related to the inflammatory conditions in the tendon sheath, different studies evaluated these variables in equine joints. Nucleated cell count recorded as <500-1000 cells/µL in normal SF of the joint and digital sheath in horses.[2,10,20,21] Inflammation increases the percentage of neutrophils and TNCC. The diagnosis of septic tenosynovitis is usually confirmed based on SF analysis. Neutrophil count often reaches more than 90% and TNCC recorded >30,000 in sepsis of SF.[1,2,8,10,12,23-25] In some other mild inflammatory condition, TNCC recorded <10000 cells/µL and the proportions of neutrophils were recorded as 4–86%.[25] Synovial fluid TNCC may not be sufficient to rule out septic arthritis;[13,26] however, some research suggests that the SF differential leukocyte count may be a better marker of joint infection.[26]

In a series of open digital flexor tendon sheath injuries in horses, a wide range of values, 1.3 to 92.6 × 10^9/L for TNCC and 33% to 98% for neutrophils were recorded.[1,27] Synovial cell count of the digital sheath in horses with and without DDFT lesions was not different.[28] A small number of TNCC and less than 10% neutrophils in the present study may consider as a sign of noninfectious nature or mild inflammation of this distension, which is supported by an increase in the count of TNCC, neutrophil, lymphocyte, and monocyte counts in DFTS.

The amount of SAA in SF is a good indicator of septic arthritis and inflammation.[23,29,30] Some studies indicated that serum and articular concentration of synovial fluids SAA didn't change after synovitis, arthroscopic lavage, and repeated injection of amikacin,[10,24,31] or a slight increase in humans' osteoarthritis, in serum and synovial fluid SAA concentrations was observed compared to the healthy control group.[23] In contrast, in other studies, SAA concentrations increase in serum and SF in septic condition[14,23,29]. SAA concentration can be used as a differentiator of infectious versus non-infectious diseases.[14] In the present study, distention of DFTS did

Table 2. Median and Percentiles (Q_1, Q_3) or Median (minimum–maximum) of biochemistry and cytology parameters in score SE.

	Score0 Median (Q_1, Q_3) No	**Score1 Median (Q_1, Q_3) No**	**Score2 Median (Q_1, Q_3) No**	**Score3 Median (Q_1, Q_3) No**	**Score4 Median (Q_1, Q_3) No**	***p* value**
D-dimer mg/l	19.30 (17.95, 20.00) 8	20.00 (3.68, 20.00) 6	20.00 (17.00, 20.00) 11	20.00 (20.00, 20.00) 9	20.00 (14.08, 20.00) 6	0.382
SAA µg/ml	0.89 (0.86, 0.98) 8	0.93 (0.86, 1.01) 6	0.91 (0.76, 1.07) 11	1.05 (0.97, 1.14) 9	0.86 (0.82, 0.99) 6	0.063
Hp µg/ml	38.85 (35.25, 40.09) 8	35.56 (33.71, 37.62) 6	35.56 (34.74, 37.21) 11	38.44 (36.10, 39.68) 9	33.71 (33.40, 41.32) 6	0.187
Lymphocytes cells/µl	85.00[a] (45.00-95.00) 7			291[b] (67.00-470.00) 9		0.001
Monocytes cells/µl	6.00 (1.00- 14.00) 7			9.00 (1.00- 14.00) 9		0.252
Neutrophils cells/µl	0.00[a] (0.00- 10.00) 7			8.00[b] (0.00- 32.00) 9		0.023
TNCC cells/µl	0.00[a] (0.00- 100.00) 8			300.00[b] (0.00- 500.00) 9		0.002

Values in tables with different superscript letters (a, b) in each row are significantly ($p \leq 0.05$) different.

Table 3. Median and Percentiles (Q_1, Q3) or Median (minimum–maximum) of biochemistry and cytology parameters in score DS.

	Score0 **No**	**Score1** **No**	***p* value**
D-dimer (mg/l)	20.00 (19.00, 20.00) 23	20.00 (19.90, 20.00) 13	0.77
SAA (µg/ml)	1.00 (0.91, 1.09) 23	0.91 (0.86, 1.04) 13	0.253
Hp (µg/ml)	37.21 (35.56, 39.26) 23	34.74 (33.50, 38.24) 13	0.92
Lymphocytes cells/µl	324.50 (67.0- 6601.0) 18	281.00 (79.00- 432.00) 6	0.28
Monocytes cells/µl	19.50[a] (4.00- 1224.00) 18	5.00[b] (0.00- 60.00) 6	0.033
Neutrophils cells/µl	7.50 (0.00- 253.00) 18	13.00 (4.00- 60.00) 6	1.00
TNCC cells/µl	400.00 (0.00- 8050.00) 18	350.00 (100.0- 450.0) 7	0.27

Values in tables with different superscript letters (a, b) in each row are significantly ($p \leq 0.05$) different.

not show a significant increase in the concentration of SAA in SF that may be an indication of less prominent inflammation. Significant reduction of SAA concentration in the serum of study versus control group is in contrast to previous studies that may be related to SAA3 produced by healthy tissue in horses.[30] Also, it could be associated with latent subclinical conditions.[32] SAA concentration of serum in the current study was in the normal range that reported as 0.5 to 50 mg/L in healthy horses.[31,32]

The serum Concentration of SAA was not significantly different from SF in the study group and was significantly higher in the control group. The relatively higher concentrations of SAA in serum compared to SF were reported in the previous study.[31] Synovial fluid SAA concentration rises slower than serum in horses.[2]

The specificity of SAA concentration is similar to TNCC, but its sensitivity is higher. In acute stages of infection, initially, TNCC may decrease as a result of margination, followed by a cytokine-mediated increase over the next 36 hours. High TNCC following treatment, often concomitant with normalization of SAA concentration and resolution of disease,[14] that may be the case in the present study.

Haptoglobin is recognized in SF of healthy horses[33] but there is very little information about the Hp changes in inflamed joint in horses and other species.[12] Increasing the concentration of Hp in the blood requires a strong stimulus.[33] A higher level of Hp was observed in samples with clinical signs that lasted for 7 days or longer, which makes it a potential tool for monitoring long-term inflammation.[17] However, some studies show Hp increase in serum and SF of arthritic horses,[12,17,32] with a difference between concentrations of serum Hp and articular SF.[17] The acute phase response can vary between animals faced with the same challenge reported that animals with the same hoof injuries presented high or undetectable serum concentrations of Hp[33].

In the present study, SAA and Hp concentrations of serum and SF in the study group was low.[8] Suppressed inflammation at the time of sampling may result in low SAA and Hp concentrations cause SAA production occurs only in response to active inflammation.[23,24]

Increased D-dimer concentrations in SF and decrease to the baseline in a short time from infected joints of the horses by a sharp increase in fibrinolysis activity were reported.[12,18,21] Non-significant difference of D-Dimer concentration of DFTS synovial fluid and serum in both groups may be a result of early sampling time because fibrinolysis occurs quite late during the joint inflammation,[12] or maybe the sampling time was after decreasing d-dimer concentration. Infectious inflammation increases the fibrinolysis,[12,20] while in clinical examinations, there was no sign of infection in this region. The cut-off concentration of synovial D-dimer in horses with the joint disease was detected as 20 mg/L.[21] Although the Synovial fluid D-dimer concentration was significantly higher than serum in both groups, results were recorded close to the border of this cut-off point.

In humans with joint disorders, synovial D-dimer concentrations were at least 10-fold higher than the concentrations in the plasma. Comparing synovial D-dimers of septicemic foals with polyarthritis and healthy foals with septic arthritis to plasma of very sick foals showed synovial concentrations were at least 100 to 150-fold higher than the plasma D-dimer concentration. Synovial D-dimer concentrations in adult horses with non-septic joint diseases were also much higher than the reported concentrations in plasma of very sick horses.[12]

However, to find out the changes related to tissue destruction or degeneration in diseases, the markers should have been specific to tendon and/or tendon sheath matrices. Cartilage oligomeric matrix protein (COMP) may be used as a prognostic marker in

rheumatoid arthritis and osteoarthritis in human, tendon injury and different articular cartilage lesions in horses.[34,35] Aggrecan accumulation also is reported as a major feature of incomplete healing in equine tendon and skin,[36] and other study detected aggrecan gene expression in different Miniature horse genetic articular disorders.[37]

As a result measuring other marker like COMP and agreecan may help in better diagnosis of tissue damages and its complications like inflamation may help in better diagnosis and prognosis of the condition that should evaluate in a control study.

Tendon sheath effusion scores (SE) in response to injection and medication prescription recorded from 0 to 3 with a median score of 0[8]. In this study, the SE score was from 0 to 4. In score 3 of SE amounts of TNCC, lymphocytes and neutrophils increased in comparison to healthy horses (score 0), indicating DFTS inflammation. By increasing SE score inflammatory reactions increased. In the present study, DS score was from 0 to 3, and sampling was done easily. Needle displacement was less than twice during sampling (score 1), a significant increase in synovial fluid monocyte count was observed. However, no increase in the amount of neutrophil is an indicator of the severity of inflammation, in this case, it isn't justifiable. While the effect of repeated arthrocentesis on cytologic analysis of SF in healthy horses shown that the total WBC count and percentage of lymphocytes, eosinophils, and neutrophils did not change over time but a variation over time was observed for monocytes and total protein concentration.[38]

Due to the increase in amounts of TNCC, neutrophil in the SF, which indicate inflammatory conditions, it can be concluded that the DFTS was inflamed, but since concentrations of SAA and D-Dimer, which indicate infectious inflammatory conditions,[20,23,29,30] do not change in SF, DFTS with distension has non-infectious inflammation or acute inflammation; no ulcer at the site or previous injection in DFTS confirm the hypothesis.

Absolutely, communication between inflammatory biomarker and severity of injury will be useful. But findings from synovial scoring did not show any corrlation between inflammatory biomarker and severity of injury. More samples and details of severity and onset of injury may be effective in determining communication in this regard.

Inflammation of DFTS was evaluated using qualitative scoring system by palpation. Adding other criteria for severity and inflammation such as color Doppler or power Doppler ultrasonography will be helpful in future studies.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Steel CM. Equine synovial fluid analysis. *Veterinary Clinics of North America: Equine Practice.* 2008;24:437-454.
2. Forsyth S. Equine synovial fluid analysis. *Vet script*. 2018;52-54.
3. Malark J, Nixon A, Skinner K, Mohammed H. Characteristics of digital flexor tendon sheath fluid from clinically normal horses. *American Journal of Veterinary Research*.1991;52:1292-1294.
4. Hassel DM, Stover SM, Yarbrough TB, Drake CM, Taylo KT. Palmar-plantar axial sesamoidean approach to the digital flexor tendon sheath in horses. *Journal of the American Veterinary Medical Association*. 2000;217:1343-1347.
5. Wright I, McMahon P. Tenosynovitis associated with longitudinal tears of the digital flexor tendons in horses:a report of 20 cases. *Equine Veterinary Journal.* 1999;31:12-18.
6. Agass R, Dixon J, Fraser B. Computed tomographic contrast tenography of the digital flexor tendon sheath of the equine hindlimb. *Veterinary Radiology and Ultrasound*. 2018;59:279-288.
7. Fortier L. Indications and techniques for tenoscopic surgery of the digital flexor tendon sheath. *Equine Veterinary Education*. 2005;17:218-224.
8. Dykgraaf S, Dechant JE, Johns JL, Christopher MM, Bolt DM, Snyder JR. Effect of intrathecal amikacin administration and repeated centesis on digital flexor tendon sheath synovial fluid in horses. *Veterinary Surgery*. 2007;36:57-63.
9. Nixon AJ. Endoscopy of the digital flexor tendon sheath in horses. *Veterinary Surgery*. 1990;19:266-271.
10. Sanchez-Teran A, Bracamonte J, Hendrick S, Riddell L, Musil K, Hoff B, Rubio-Martínez L. Effect of repeated through-and-through joint lavage on serum amyloid A in synovial fluid from healthy horses. *The Veterinary Journal.* 2016;210:30-33.
11. Hagberg L, Heinegard D, Ohlsson K. The contents of macromolecule solutes in flexor tendon sheath fluid and their relation to synovial fluid: a quantitative analysis. *The Journal of Hand Surgery: British and European Volume.* 1992;17:167-171.
12. Andreassen SM, Vinther AM, Nielsen SS, Andersen PH, Tnibar A, Kristensen AT, Jacobsen S. Changes in concentrations of haemostatic and inflammatory biomarkers in synovial fluid after intra-articular

injection of lipopolysaccharide in horses. *BMC Veterinary Research*. 2017;13(1):1-7.
13. McGillicuddy DC, Shah KH, Friedberg RP, Nathanson LA, Edlow JA. How sensitive is the synovial fluid white blood cell count in diagnosing septic arthritis? *The American Journal of Emergency Medicine*. 2007;25:749-752.
14. Belgrave RL, Dickey MM, Arheart KL, Cray C. Assessment of serum amyloid A testing of horses and its clinical application in a specialized equine practice. *Journal of the American Veterinary Medical Association.* 2013;243:113-119.
15. Satue K, Calvo A, Gardon J.C. Factors influencing serum amyloid type A (SAA) concentrations in horses. *Open Journal of Veterinary Medicine*. 2013;3:58.
16. Witkowska-Piłaszewicz O, Zmigrodzka M, Winnicka A, Miskiewicz A, Strzelec K, Cywinska A. Serum amyloid A in equine health and disease. *Equine Veterinary Journal.* 2019;51:293-298.
17. Barrachina L, Remacha AR, Soler L, Garcia N, Romero A, Vazquez FJ, Vitoria A, Alava MA, Lamprave F, Rodellar C. Acute phase protein haptoglobin as inflammatory marker in serum and synovial fluid in an equine model of arthritis. *Veterinary Immunology and Immunopathology*. 2016;182:74-78.
18. Qin L, Li F, Gong X, Wang J, Huang W, Hu, N. Combined measurement of D-dimer and C-reactive protein levels: Highly accurate for diagnosing chronic periprosthetic joint infection. *The Journal of Arthroplasty.* 2020;35:229-234.
19. Xu H, Xie J, Huang Q, Lei Y, Zhang S, Pei F. Plasma fibrin degradation product and D-dimer are of limited value for diagnosing periprosthetic joint infection. *The Journal of Arthroplasty.* 2019;34:2454-2460.
20. Ribera T, Monreal L, Armengou L, Rios J, Prades M. Synovial fluid D-dimer concentration in foals with septic joint disease. *Journal of Veterinary Internal Medicine*. 2011;25:1113-1117.
21. Ribera T, Monreal L, Delgado M, Rios J, Prades M. Synovial fluid D-dimer concentration in horses with osteochondritis dissecans and osteoarthritis. *Veterinary and Comparative Orthopaedics and Traumatology*. 2013;26:54-60.
22. Stokol T, Erb HN, De Wilde L, Tornquist SJ, Brooks M. Evaluation of latex agglutination kits for detection of fibrin (ogen) degradation products and D-dimer in healthy horses and horses with severe colic. *Veterinary Clinical Pathology.* 2005;34:375-382.
23. Jacobsen S, Thomsen M. H, Nanni, S. oncentrations of serum amyloid A in serum and synovial fluid from healthy horses and horses with joint disease. *American Journal of Veterinary Research*. 2006;67:1738-1742.
24. Sanchez Teran A, Rubio-Martinez LM, Villarino NF, Sanz, MG. Effects of repeated intra-articular administration of amikacin on serum amyloid A, total protein and nucleated cell count in synovial fluid from healthy horses. *Equine Veterinary Journal.* 2012;44:12-16.
25. Koenig RD. Synovial Fluid Analysis of the Horse. *In: Veterinary Cytology.* Leslie C, Sharkey MJR, Seelig D. ed. John Wiley & Sons. 2021.
26. Baran S, Price C, Hak DJ. Diagnosing joint infections: synovial fluid differential is more sensitive than white blood cell count. *European Journal of Orthopaedic Surgery & Traumatology*. 2014;24:1469-1474.
27. Steel CM. Equine synovial fluid analysis. *Veterinary Clinics of North America: Equine Practice.* 2008;24:437-454.
28. Huggons N, Bell R, Puchalski S. Characterization of digital sheath fluid in horses with deep digital flexor tendinopathy. *The Australian Equine Veterinarian*. 2010;29:82-82.
29. Robinson CS, Singer ER, Piviani M, Rubio-Martinez LM. Are serum amyloid A or D-lactate useful to diagnose synovial contamination or sepsis in horses? *The Veterinary Record*. 2017;181:425.
30. Jacobsen S, Niewold TA, Halling-Thomsen M, Nanni S, Olsen E, Lindegaard C, Andersen PH. Serum amyloid A isoforms in serum and synovial fluid in horses with lipopolysaccharide-induced arthritis. *Veterinary Immunology and Immunopathology*. 2006;110:325-330.
31. Ludwig EK, Brandon Wiese R, Graham MR, Tyler AJ, Settlage JM, Werre SR, Petersson-Wolfe CS, Kanevsky-Mullarky I, Dahlgren LA. Serum and synovial fluid serum amyloid A response in equine models of synovitis and septic arthritis. *Veterinary Surgery.* 2016;45:859-867.
32. Hulten C, Gronlund, U, Hirvonen J, Tulamo RM, Suominen M, Marhaug G, Forsberg M. Dynamics in serum of the inflammatory markers serum amyloid A (SAA), haptoglobin, fibrinogen and α2-globulins during induced noninfectious arthritis in the horse. *Equine Veterinary Journal.* 2002;34:699-704.
33. Filippo PAD, Lannes ST, Meireles MA, Nogueira AF, Quirino CR. Concentrations of acute-phase proteins and immunoglobulins in serum and synovial fluid in clinically healthy heifers and steers. *Pesquisa Veterinária Brasileira.* 2019;39:388-392.
34. Smith RKW, Heinegård D. Cartilage oligomeric matrix protein (COMP) levels in digital sheath synovial fluid and serum with tendon injury. *Equine Veterinary Journal*. 2010;32 (1):52-58.
35. Skioldebrand E, Lorenzo P, Zunino L, Rucklidge GJ, Sandgren B, Carlsten J, Ekman S. Concentration of collagen, aggrecan and cartilage oligomeric matrix protein (COMP) in synovial fluid from equine middle carpal joints. *Equine Veterinary Journal*. 2001;33(4):394-402.
36. Plaas A, Sandy J, Liu H, Diaz M, Schenkman D, Magnus R, Bolam-Bretl C, Kopesky P, Wang M, Galante J. Biochemical identification and immunolocalizaton of

aggrecan, ADAMTS5 and inter-alpha-trypsin–inhibitor in equine degenerative suspensory ligament desmitis. *Ortopedic Research.* 2011;29(6):900-906.

37. Basso R, Andred D, Alves C, Lufer R, Borges A, Oliveira-Filho P. Aggrecan, IL-1β, IL-6, and TNF-α profiles in the articular cartilage of miniature horses with chondrodysplastic dwarfism. 2021;103.
38. Witkowska-Piłaszewicz O, Żmigrodzk, M, Winnicka A, Miśkiewicz A, Strzelec K, Cywińska A. Serum amyloid A in equine health and disease. *Equine veterinary journal.* 2019;51:293-298.