THE IMPORTANCE OF SYNOVIAL FLUID IN THE DIAGNOSIS OF EQUINE ARTHROPATIES

IMPORTANŢA LICHIDULUI SINOVIAL ÎN DIAGNOSTICUL ARTROPATIILOR LA CABALINE

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ABSTRACT

The articular system, as a component of the locomotor system, is of particular importance as it is responsible for the absorption and distribution of forces acting on the limbs. Affecting this system can lead to significant economic losses, so it is imperative to use non-invasive, precise and economic techniques to establish a diagnosis as early as necessary for better joint lesion management.

In this study, we performed a clinical and paraclinical investigation of articular system, including an examination of the synovial fluid collected from 12 horses with different grades of lameness. The examined horses were divided into two groups based on the lameness grade established according to the lameness scale implemented by the American Association of Equine Veterinarians. Thus, in group no. 1 there were included 6 horses with 1st grade of lameness, and group no.2 was made up of 6 horses with 3 and 4 grade of lameness. The results revealed the presence of cytological, biochemical and haematological modifications of the synovial liquid collected from the two groups of horses, but no association could be established between the values of the determined biochemical parameters and the clinical manifested signs.

Consequently, we recommend that the synovial fluid exam, together with clinical and radiological examination, become a common practice in the diagnosis of joint diseases in horses.

Keywords: horse, arthropathies, lameness, diagnostic

REZUMAT

Sistemul articular, ca parte componentă a aparatului locomotor prezintă o importanță deosebită, acesta fiind responsabil de absorbția și distribuția forțelor care acționează asupra membrelor. Afectarea acestui sistem poate determina apariția unor pierderi economice importante, fiind imperativă utilizarea unor tehnici non-invazive,precise și cât mai puțin costisitoare în vederea diagnosticării precoce a leziunilor articulare.

În acest studiu s-a efectuat o investigare clinică și paraclinică a sistemului articular, respectiv o examinare a lichidului sinovial recoltat de la 12 cai cu grade diferite de șchiopătură. Caii examinați au fost împărțiți în două loturi pe baza gradului de șchiopătură stabilit conform scalei de gradație a șchiopăturilor implementată de către Asociația Americană a Veterinarilor de Ecvine. Astfel, în lotul 1 au fost incluși 6 cai cu șchiopătură de gradul 1, iar lotul 2 a fost alcătuit din 6 cai cu șchiopătură de gradul 3, respectiv 4. Rezultatele obținute au relevat prezența unor modificări citologice, biochimice și hematologice ale lichidului sinovial recoltat de la cele două loturi de cai, dar nu s-a putut stabili o asociere între valorile parametrilor biochimici determinați și semnele clinice manifestate.

Ca urmare a acestor rezultate recomandăm ca examenul lichidului sinovial, alături de examinarea clinică și radiologică, să devină o practică curentă în diagnosticul afecțiunilor articulare la cabaline.

Cuvinte cheie: cal, artropatii, șchiopătură, diagnostic

The synovial fluid is a fundamental component of the joints, tendons and bursae, being considered as a biological lubricant and an important source of nutrients for the intra-articular tissues (3; 16). This is a blood plasma ultra-filtrate that differentiates from it by several elements: it has fewer proteins, high content in hyaluronic acid, and the cell population is limited, normally not exceeding 500 cells/mm^3 (10). The cells found in the synovial fluid collected from the healthy animals are lymphocytes and macrophages (16; 17). In the literature, there are described three mechanisms responsible for the synovial fluid formation: ultra-filtration of the blood plasma at the capillary level, its elaboration at the level of synovial interstitial

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fluid, directly from the synovial cytes type B, respectively the passage from the lymphatic vessels to the inside of the joint (3; 4; 6).

Among the musculo-skeletal diseases of the equines, joint diseases and lesions make up a significant number of medical cases that lead to lameness and, implicitly, affect the mechanic performance of the horses (12; 14). The key to treating these joint problems is choosing some early and accurate diagnostic methods. The clinical examination of these patients provides insufficient information to establish the certainty of the diagnosis, and the imaging methods are costly and require competent, specialized and well-trained staff. The *gold standard method* for establishing a diagnosis of certainty in intraarticular soft tissue disorders such as cartilage, ligaments, menisci, synovial membranes is arthroscopy, a technique that requires a lot of attention from the examiner, as it is accompanied by a multitude of intraoperative and postoperative complications (5; 9).

A potential early examination and diagnosis method of the intra-articular inflammation can also be the synovial fluid examination (8).

In this study, the radiological and synovial fluid evaluations were used as complementary examinations techniques in the establishment of a diagnosis in horses with varying grades of lameness.

The aim of our study was to set up a working protocol in the diagnosis of articular disorders, which will be the basis for localizing the lesion, the nature and the etiology of the pathological process.

MATERIALS AND METHOD

Animals.

The biological material used for this research was made up of 12 sterile synovial fluid samples collected from 12 horses with articular clinical manifestations. The age of the examined horses (3 female horses, 3 castrated male horses and 7 uncastrated male horses) ranged from 6 months to 13 years. The samples were collected from the stifle, hock and fetlock joints. The horses included in the study were hospitalized in the Clinic of Equine of the Faculty of Veterinary Medicine from Cluj-Napoca.

The research was conducted in the Department of Medical Imaging, The Clinic of Equine, The Microbiology Laboratory, The Clinic and Biochemistry Laboratory from the Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania.

Study protocol.

For the joint examination, the following classical semiological methods were used: inspection, palpation, percussion, complemented by articular puncture and radiological evaluation.

The inspection was carried out from the front, back and side, conducting both a distant as well as a close examination. Following the exam, data on the dynamics of joint movement, joint volume, possible protrusions of the suprapatellar recess and data on periarticular areas were obtained. Also, the integrity of the covering tissues has been thoroughly examined.

The emphasis was on the mobility of the joints by performing flexion and extension movements in order to determine the grade of arthropathy. The grade of lameness was determined by the scale of the lameness developed by the American Association of Equine Practitioners (AAEP). According to this scale in horses there are 5 grades of lameness:

- grade 0: Lameness not perceptible under any circumstances;
- grade 1: Lameness is difficult to observe and is not consistently apparent;
- grade 2: lameness is observable in certain circumstances (specific surfaces where the horse is examined: under saddle, circling, inclines, hard surface) is difficult to observe at a walk or when trotting in a straight line;
- grade 3: Lameness is consistently observable at a trot under all circumstances
- grade 4: Lameness is obvious at a walk;
- grade 5: Lameness produces minimal weight bearing in motion and/or at rest or a complete inability to move.

Following the palpation of the joints, data was obtained on: local temperature, sensitivity, resistance and mobility in flexion and extension movements, respectively, fluctuations and voltages of the synovial fluid. The radiological examination and interpretation of the obtained radiographic images complied with the standard screening protocol.

Gram staining method was used to stain the synovial fluid smears.

The data obtained from the clinical and radiological examination together with the laboratory results were systematized into individual examination sheets, then analyzed and interpreted.

Articular puncture.

Synovial fluid samples were collected thorough arthrocentesis from the forefoot fetlock, stifle and hock

Table 1

The characteristics of horses included in the study

Variable	1	2	3	4	5	6	7	8	9	10	11	12
Age	6m	7y	13y	5y	6y	8y	9y	10y	7y	8y	7y	4y
Name	Athos	Alto	Venusz	Pisti	Rondador	Fani	Valto	Fulger	Pluto	Vera	Sarkazi	Charlatan
Sex	Mn	Mn	F	Mc	Mn	F	Mn	Mc	Mn	F	Mc	Mn
Grade of lameness	1	3	4	3	1	3	1	3	4	1	1	1
Joint	L. h.	L. h.	R. h.	R. h.	R. h.	L. h.	R. s.	L. f. f	R. h.	L. f. f	L. h	R. f. f

Joint - Joint where the puncture was made; **Mn**-uncastrated male horse; **F**-female horse; **Mc**-castrated male horse; **L.h**.-left hock; **R.h**.- Right hock; **R.s**.-right stifle; **L.f.f**. -left forefoot fetlock; **R.f.f**.-right forefoot fetlock; **y**-year; **m**-month

joints. The arthrocentesis of the metacarpal-sesamophalange joint was performed on the side of the fetlock through the thickness of the metacarpal-sesamoidian collateral ligament. The limb was lifted off the ground and the joint flexed to increase the space between the metacarpal and the sesamoid bones. The space thus formed can be identified in palpation as a depression located between the bones. The arthrocentesis of the tibio-tarso-metatarsal joint was carried out at the suprapatellar bursa level of the talocrural joint side of the synovium, which is subcutaneously palpable. The arthrocentesis of the femoral-tibial-patellar joint was performed at the femoral-patellar joint level.

The collection of synovial fluid was performed in compliance with the aseptic and antiseptic standards.

The materials used for collection were represented by biochemistry sterile vacutainer tubes (red) and EDTA tubes (purple) for cell morphology, 18 gauge pink sterile needles compatible with both Luer slip and Luer lock systems; syringes for synovial fluid collection, wound dressings and adhesive plasters. Asepsis was performed with sanitary alcohol and iodine solutions (Betadine).

The haematological and biochemical examination of synovial fluid samples was performed in the clinical laboratory, considering the following parameters: Leukocyte (WBC), red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), platelets (PLT), total proteinemia, globulins, glucose, aspartate aminotransferase (AST), alkaline phosphatase (PAL), lactate dehydrogenase (LDH) and lactate.

The reference values for the evaluated biochemical and haematological parameters were:

WBC:5.0-10.0*10^3/mm^3; HGB: 9-18 g/dl; PLT: 100-400*10^6/mm^3; Pt: 2 g/dl; Glucose: 50-90 mg/dl; ASAT: 240 U/l; PAL: 150-230 U/l; LDH: 150-400 U/l; Lactate: 9-16.2 mg/dl.

Statistical analysis.

The analysis of the data was performed using R 2.15.1 software. The Kolmogorov-Smirnov test was used to calculate the normal distribution of the variables. Continuous and non-normally distributed data were expressed as medians [25th percentile, 75th percentile]. The Kruskal Wallis Test was used to compare the groups of variables. A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSIONS

Table 1 presents the characterization of the horses that were examined and the difference in the grade of lameness based on the characteristics of the synovial fluid is presented in Table 2.

The horses admitted to the medical examination were divided into two groups based on the lameness scale established, developed and implemented by The American Association of Equine Practitioners (AAEP).

The first group consisted of 6 horses that presented a grade 1 of lameness (n = 6), and the second group consisted of 6 horses showing grade 3 and 4 of lameness.

The data from Table 2 on the two groups of horses that were examined, presenting grade 1 or grade 3 and 4 of lameness, show different values in the biochemical and haematological parameters. For grade 1 of lameness, the values of the analyzed biochemical and haematological parameters are higher compared to those values of horses diagnosed with Grade 3 and 4 of lameness but this difference is not statistically significant. These results are confirmed by the smear examination that provides additional information about the existence of neutrophils and lymphocytes for the two horses diagnosed with grade 1 of lameness, cases no. 5 and 7 (Fig. 1).

Table 2

Differences between different grades of lameness in horses in terms of synovial fluid biochemical and hematological results

Parameter	Lameness grade 1 (n=6)	Lameness grades 4+3 (n=6)
WBC ($*10^3/mm^3$)	0.35[0.30, 0.40]	0.35[0.27, 0.90]
HGB (g/dl)	0.00[0.00, 3.2]	0.00[0.00, 1.27]
PLT ($*10^3/mm^3$)	40.50[16.25, 55.00]	31.00[10.00, 76.00]
Pt (g/dl)	1.94[1.57, 2.50]	1.36[1.24, 2.30]
Albumin (g/dl)	0.99[0.73, 1.25]	0.79[0.66, 0.99]
Globulin (g/dl)	1.00[0.35, 1.68]	0.63[0.37, 1.52]
Glucose (mg/dl)	140.65[136.20, 156.52]	143.25[121.20, 153.62]
AST (U/l)	213.95[208.82, 223.77]	208.10[179.82, 221.90]
ALP (U/l)	188.95[182.77, 195.35]	178.00[152.60, 208.65]
LDH (U/l)	352.05[289.10, 407.27]	358.50[314.67, 394.70]
Lactate (mg/dl)	14.94[14.50, 15.40]	14.15[13.85, 14.76]

Thus, in the case of horse no. 5, the inflammatory profile (presence of neutrophils) is also accompanied by a value above the physiological limit of LDH (Table 3). This increased value suggests the existence of skeletal lesions, lesions that mean degeneration. In addition to this increased LDH value, an increase in the AST enzyme and total protein content (Table 3) was also revealed. An increase in the number of neutrophils and lymphocytes in the synovial fluid smear was also noted in case no. 7, these elements show the existence of an inflammation with possible bacterial contamination in the stifle joint. Moreover, the presence of lymphocytes in the synovial fluid collected from the two cases indicates the existence of chronic inflammation. Thus, in both situations one can discuss the existence of an inflammatory process at the level of the examined joints, but without its exteriorization, the horses did not show clinically visible lameness, only a certain discomfort at a walk. All these elements explain the blood and biochemical data for horses diagnosed with grade 1 of lameness. The data obtained by haematological and biochemical changes of the synovial fluid collected from horses with various joint problems, which implicitly lead to lameness, have been highlighted in other studies conducted so far (1,2,11). Thus, in these studies, a change in the total leukocytes count, particularly neutrophils and monocytes, was shown in the case of the horses diagnosed with acute arthritis, an insignificant increase in the number of leukocytes predominantly monocytes in horses diagnosed with chronic arthritis. There have been identified biochemical changes, such as an increase of the values for the AST, ALT, LDH, total protein content respectively. An explanation for the increased activity of these enzymes can be attributed to their generous release of the high levels of leukocytes present in the synovial fluid of horses with joint problems (1; 7; 19).

The horses in group 2 diagnosed with grade 3 and 4 lameness did not show significant changes in the synovial fluid smear (Fig. 2 A, C), the haematological and biochemical examination was insignificant.

Important changes were highlighted by the radiological examination and its results reported signs associated with the arthrosis, as well as a slight distension of the hock joint bones, elements that helped explain the diagnosed lameness (Fig. 2 B, D).

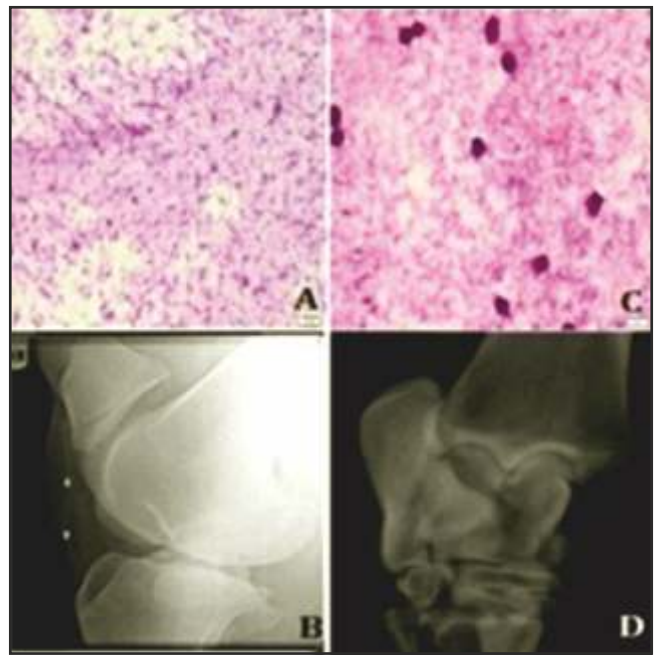

Fig. 1. Examination of synovial fluid smear and radiological examination for case no. 7- Valto (A, B) and 5- Rondador (C, D). We can observe the presence of lymphocytes and neutrophils in the smear examination (A, C) for the two cases diagnosed with grade 1 arthropathy.
The radiological examination of the joints for the two cases shows a reaction to the insertion of the medial femoral-patellar ligament (B – for case 7) and a disappearance of the interosseous articular space in the tibio tarso metatarsal joint (D) – for case 5.

Another characteristic of the examined horses was the AST and lactate values that were close to or above the upper limit. The explanation for these values is due either to the presence of small muscular lesions or to the existence of a healthy but overloaded muscle tissue because the damaged limb is no longer used.

Table 3

Variation of different biochemical parameters in the synovial fluid

	Athos	Charlatan	Pisti	Rondador	Alto	Pluto	Sarkozi	Fani	Vera	Valto	Fulger	Venusz
AST (U/l)	194,2	95,6	198,5	225,8	232,7	213,7	39,3	218,3	223,1	214,2	217,7	134,3
Lactate (mg/dl)	16	12,1	14	15,08	14,1	14,5	24,24	14,12	14,8	15,2	13,4	16,3
ALP (U/l)	186,3	153,7	209,7	176,1	156	191,6	81,1	208,3	185	206	142,4	160,6
LDH (U/l)	359,8	271,8	361,6	431,8	324,1	289,1	125,1	392,7	399,1	344,3	400,7	286,4

The values for LDH and ALP above the normal upper limit or close to the upper limit bend the diagnosis towards localized bone disease (18) (Table 3).

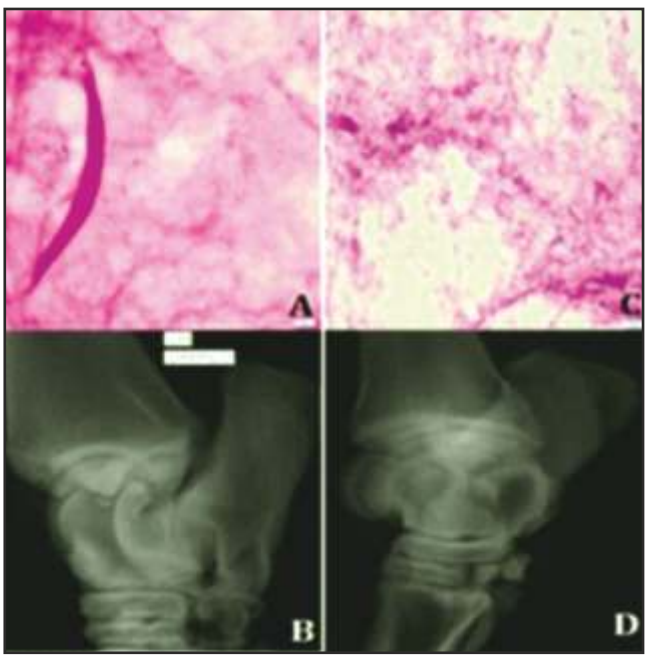

Fig. 2. Examination of synovial fluid smear and radiological examination for case no. 2 - Alto (A, B) and 4 - Pisti (C, D), diagnosed with Grade 4 and 3 lameness, respectively. The examination of synovial fluid smear (A, C) reveals the lack of cells in both smears and the presence of a fibrin network. The radiological examination of the hock joint in Case 2 reveals signs of arthritis (B) and the disappearance of the interosseous articular space, with a mild distension of the hock in case no. 4 (D).

CONCLUSIONS

■ The synovial fluid examination in the two groups of examined horses revealed both cytological and biochemical changes, as well as haematological changes. The values of the analyzed biochemical parameters were not associated with the degree of lameness, i.e. the clinical manifestations. Thus, the values of biochemical parameters were in range of physiological limits in the case of horses with grade 3 and grade 4 of lameness and were exceed the physiological limits in the case of horses with a grade 1 of lameness.

■ We recommend that the synovial fluid exam become a common practice in the diagnosis of joint diseases in horses, along with clinical and radiological examination.

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