RESEARCH ARTICLE

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Stem Cell Therapy in the Treatment of Elbow Joint Osteoarthritis in Dogs

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ABSTRACT

Osteoarthritis is a common disease in dogs that cause chronic pain, stiffness and can greatly reduce the quality of life. Standard treatment mostly is still symptomatic and is based on pain relief, without any disease-modifying effect and the side effects of painkillers can occur relatively common. The aim of this study was to determine do single intra-articular allogeneic stem cell injection gives improvement on patient well-being and have disease remodeling effects. Ten dogs with unilateral elbow osteoarthritis were selected. In osteoarthritic joint single stem cell intra-articular injection was made. Treatment was assessed by using stance analyzer to detect weight distribution, goniometry to measure range of motion, radiological examination to assess osteophyte size and size of subtrochlear sclerosis, joint fluid and blood sample analysis was made before and six months after treatment and once a month animal owner questionnaires were made. There was beneficial changes in weight distribution, range of motion, in size of osteophyte and subtrochlear sclerosis but not statistically significant. But in patient well-being observations from animal owner questionnaires there was significant improvements.

Keywords: CaniCell, canine stem cells, elbow join, osteoarthritis, Medrego.

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I. Introduction

Osteoarthritis (OA) is a degenerative joint disease [1] that affects articular cartilage, subchondral bone, synovial membrane, and ligaments [2]. OA is observed in approximately 5% of the dog population [3]. OA is the most common cause of chronic pain in dogs [4]. OA is a complex disease that alters the homeostasis of joint tissue, leading to a predominance of degenerative processes [5]. Recently, many studies have shown that synovial inflammation characterized by immune cell infiltration, such as dendritic cells, macrophages, natural killer cells, and T cells, precedes structural cartilage changes. Joint cartilage has limited healing potential due to the absence of direct contact with blood vessels and innervation [6]. Pain and joint capsule fibrosis may be associated with reduced joint range of motion (ROM) [7]. Current treatment of OA is focused on pain relief and symptom control [8]. Nonsteroidal anti-inflammatory drugs (NSAIDs) have historically been the choice of drugs for the management of OA because of their analgesic and anti-inflammatory properties [9] but NSAIDs often do not provide complete pain relief [10] and have been associated with renal, gastrointestinal, and hepatic toxicity [3]. Cartilage repair has often been an area of interest [1] and surgical procedures have not been able to effectively repair articular cartilage [6]. And unlike drug therapy, cell therapy has a trophic function by attaching endogenous cells to the injured area [10]. Stem cells are defined as undifferentiated cells capable of transforming into differentiated cells and regenerating tissues [11]. Mesenchymal stem cells (MSCs) release immunomodulatory factors that allow them to avoid repulsive mechanisms to exert their therapeutic effects [12]. MSC transplantation has not been associated with any adverse effects and does not appear to be associated with an increase in malignancies [13].

The aims of this study were to evaluate whether an intra - articular MSC injection has a long - term beneficial effect on weight bearing, joint range of motion, radiologically observable OA signs, the composition of synovium, lameness, and thus improves the quality of life and is safe to use.

II. Materials and Methods

The study was performed at the small animal veterinary clinic of the Latvia University of Life Sciences and Technologies from December 2018 to February 2020. Ten dogs with unilateral OA of the elbow were selected for the study. Patients were selected on the basis of clinical and radiological examinations. The study plan was for the selected dogs to undergo examinations and questionnaires of the animal owner before stem cell therapy, and to repeat the examinations and questionnaires once a month for a total of six months. Data of the animals were collected such as age, breed and OA-affected elbow joint (right or left, based on previous examinations). In the animal owner questionnaire, the owner had to evaluate in a 5-point system lameness (1 – is not lame, 2 – sometimes, 3 – constantly a little bit, 4 –

constantly, 5 – not walking on the affected leg), pain (1 - without pain, 2 – sometimes minimal to mild pain, 3 – constantly minimal to mild pain, 4 – constantly moderate pain, 5 – constantly severe pain), stiffness / functional ability (1 – without, 2 – sometimes, 3 – constantly minimal to mild stiff, 4 – constantly moderate stiff, 5 – severe stiff, do not want to walk), and quality of life / improvement (1 – getting worse, 2 – no changes, 3 – mild improvement, 4 – good improvement, 5 – excellent improvement).

Initially, animal owner questionnaires were conducted and patients were examined on stance analyser 'ReHab KRUUSE E1061'. The stance analyser measures the distribution of body weight on each limb when standing and may allow for subtle differences to be seen between the outcomes of various surgical procedures and allow assessment of progression of the disease or recovery over time [14]. A minimum of 5 measurements were recorded each time this test was performed. An intravenous catheter was inserted in *v. cefalica* and blood samples for haematological and biochemical analysis were taken. Propofol was administered intravenously to sedate the animal. The sedated animal underwent a goniometric examination of both elbow joints. Goniometry is performed with a measuring device, a transparent plastic goniometer. This test can be used to quantify the amplitude of joint movements (ROM). The maximum extension angle and the maximum flexion angle of the elbow joints were determined, and the amplitude of the joint movement was calculated mathematically. The goniometer was placed on the center of the elbow joint above the axis of rotation of the joint, one arm of the goniometer was placed along the longitudinal axis of the antebrachium and the other one along the longitudinal axis of the humerus [15]. Each patient underwent an x-ray examination of both elbow joints in two projections. A mediolateral projection was made positioning the dog in lateral recumbency as well as a craniocaudal projection was made positioning the dog in ventral recumbency. Signs of OA were assessed radiographically. If observed osteophytethe on anconeal process (*proc. anconeus*) to measure its length the line along dorsal non-articular border of the anconeal process was created and distance from the line to osteophyte apex was measured. Each flexed mediolateral projection was assessed for the presence of subtrochlear sclerosis (STS). When present, STS was quantified as a percentage of the length between 2 fixed points. To measure % STS, a line was created perpendicular to the most caudal margin of the ulnar proximal metaphyseal cortex and to the most proximo-caudal aspect of the radial head (Y). The STS caudal border which constituted a subjective radiographic assessment of the junction between sclerotic and normal trabecular bone pattern was created along the line to most proximo-caudal aspect of the radial head (X). The distance X was expressed as a percentage of the total distance Y. The % STS was calculated as 100(X/Y) [16]. After radiographic examination, the site for arthrocentesis was prepared. The lateral site of the elbow was clipped (clipper blade 40#). Preparation of the injection site was made by using alcohol and chlorhexidine swabs. The 'CaniCell' stem-cell product from the company 'MEDREGO' containing 3 million cells was used. The tube with the stem cells was manually defrosted and transferred to a sterile syringe. An intra-articular injection of aspirated joint fluid (~1.0 mL) was then made to the elbow joint and the syringe was removed from the needle (the needle was kept in the joint). The syringe with the stem cell product was added to the needle and cells were injected into the joint. The syringe with a needle was removed from the joint and a slight massage on the injection site was made. The second elbow for arthrocentesis was prepared as previously described and synovium (~1.0 mL) was taken. Cytological analyses were performed of the aspirated joint fluid from both (healthy and OA affected) elbow joints. After arthrocentesis, a light pressure bandage was applied. Once a month for six consecutive months, the dogs were examined on a stance analyser scales and a questionnaire of animal owners was conducted. In the third month after stem cell therapy, additional radiographic and goniometric examinations were performed, and in the sixth month after stem cell therapy, radiographic examinations and goniometric examinations were performed, as well as blood samples for haematological and biochemical examination and arthrocentesis were also performed on both elbow joints to obtain fluid samples from the joint. The amount of type B synoviocytes, fibrin, protein, neutrophils, erythrocytes, lymphocytes and macrophages was determined by cytological analysis of the joint fluid. For data analysis and a graphical representation, the analysis data were graded in points: 0 - none, 1. - rare, 2. - slightly, 3. - moderately, 4. - much.

For data processing Microsoft excel 2016 and IBM SPSS 20.0 software were used. To obtain mean, median, percentage, minimum and maximum values descriptive statistic methods were used. The arithmetic mean was used to estimate the central trend. A standard deviation (SD) was used to estimate data dispersion. The conformity of the data obtained in the study to the normal distribution was tested by the Kolmogorov-Smirnov and Shapiro-Wilk tests. Statistical analysis was performed using a significance level of 0.05. Covariance analysis (ANCOVA) was used to determine the total change in data over the months. Monthly changes in parametric data were compared with the paired T-test. The Wilcoxon test was used to compare non-parametric data by months. For calculations to determine the statistical significance of data form animal owners questionnaires about quality of life, before stem cell therapy, at month 0, a score of '2 - no change' was used.

III. Results

In the study participated 3 Rottweilers, 2 Labrador Retrievers, 1 Newfoundland, 1 Bullmastiff, 1 Golden Retriever, 1 Welsh Corgi, 1 White Swiss Shepherd Dog but this participant, due to general health issues unrelated to stem cell therapy, withdrew. Five of the dogs were males and four were females. At the beginning of the study mean age was 34.33 months ± 27.3 SD (min 9 months, max 72 months). Mean body weight was 44.66 kg ± 16.24 SD (min 15.9 kg, max 67.0 kg). For 4 dogs right elbow was OA affected and for 5 dogs left elbow.

From measurements on a stance analyser weight on OA affected foreleg 6 months after stem cell therapy increased in 66.7 % dogs, but not significantly $p = 0.053$ (Fig. 1).

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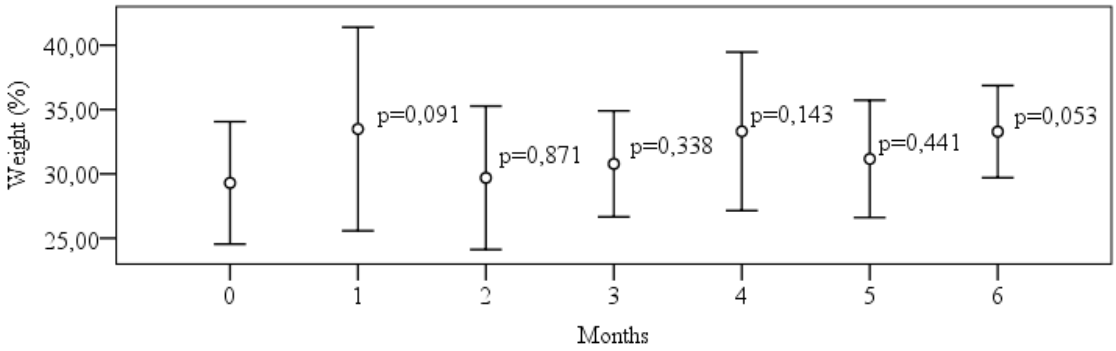

Fig. 1. Weight on the OA affected forelegs by month.

TABLE I: FLEXION ANGLE BY MONTHS

	Before therapy	3 months after therapy	6 months after therapy
Flexion angle (°) OA elbow ± SD	52.22 ± 9.46	54.44 ± 7.94	56.11 ± 11.07
Flexion angle (°) healthy elbow ± SD	52.00 ± 11.87	52.22 ± 9.02	52.33 ± 11.91

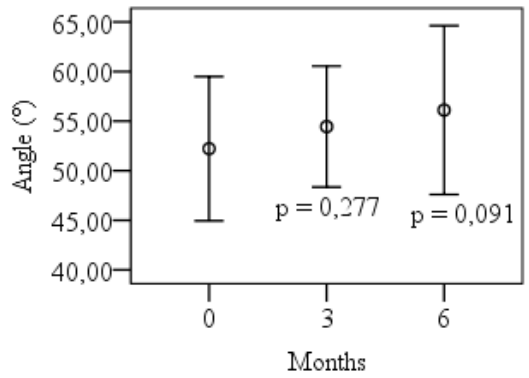

Fig. 2. Flexion angle in the OA affected elbow.

TABLE II: EXTENSION ANGLE BY MONTHS

	Before therapy	3 months after therapy	6 months after therapy
Extension angle (°) OA elbow ± SD	147.78 ± 4.99	151.44 ± 3.20	152.56 ± 3.01
Extension angle (°) healthy elbow ± SD	152.89 ± 3.53	154.33 ± 2.05	155.56 ± 3.06

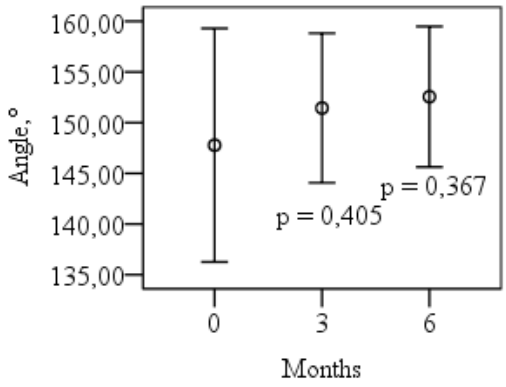

Fig. 3. Extension angle in the OA affected elbow.

TABLE III: RANGE OF MOTION BY MONTHS

	Before therapy	3 months after therapy	6 months after therapy
ROM (°) of OA elbow ± SD	97.22 ± 6.30	98.67 ± 3.92	97.56 ± 4.21
ROM (°) of healthy elbow ± SD	100.89 ± 4.61	102.11 ± 3.14	103.22 ± 3.20

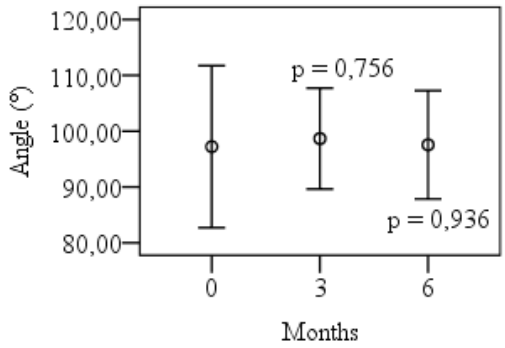

Fig. 4. Range of motion of OA affected elbow by months.

TABLE IV: SUBTROCHLEAR SCLEROSIS BY MONTHS

	Before therapy	3 months after therapy	6 months after therapy
STS % OA elbow ± SD	51.28 ± 10.01	48.87 ± 8.42	47.24 ± 9.13

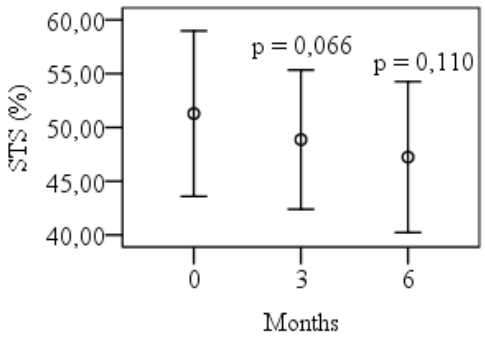

Fig. 5. Percental subtrochlear sclerosis of OA affected elbow by months.

Flexion angle 6 months after stem cell therapy decreased in 33.3 % dogs, increased in 55.6 % dogs, and remained the same in 11.1 % (Table I). No statistically significant changes were observed when compared by month before and 6 months after therapy ($p > 0.05$) (Fig. 2).

Extension angle in OA affected elbow after stem cell

therapy increased in 66.7 % dogs, but decreased in 33.3 % dogs (Table II). No statistically significant changes were observed when compared by month before and 6 months after therapy ($p > 0.05$) (Fig. 3).

ROM 6 months after stem cell therapy increased 55.6 % dogs, for 11.1 % remained the same and for 33.3 % dogs decreased (Table III). Compared data by months no significant changes were observed ($p > 0.05$) (Fig. 4).

STS % in OA affected joints decreased in 66.7 % of dogs, 11.1 % remained the same and for 22.2 % increased (Table IV). Compared data by months no significant changes were observed ($p > 0.05$) (Fig. 5).

In the radiological evaluation of osteophyte size on the anconeal process in OA affected elbow, osteophyte was absent for 33.3 % of dogs, it decreased in size in 44.4 % dogs, 11.1 % stay remained and for 11.1 % of dogs osteophyte increased in size (Table V). Statistically comparing osteophyte size by months before and after stem cell therapy no significant changes were observed ($p > 0.05$) (Figure 6).

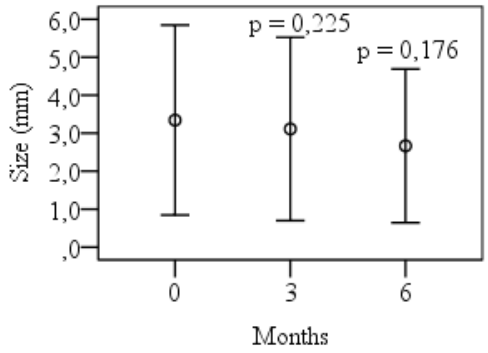

Fig. 6. Osteophyte size on anconeal process by months.

TABLE V: OSTEOPHYTE SIZE ON ANCONEAL PROCESS BY MONTHS

	Before therapy	3 months after therapy	6 months after therapy
Osteophyte size (mm) ± SD	3.49 ± 3.45	3.11 ± 3.14	2.67 ± 2.63

In blood tests, haematological analysis (Table VI) and biochemical analysis (Table VII), no significant deviations were observed, neither before nor 6 months after stem cell therapy. Values remain within normal limits, except before therapy slight hypocalcaemia by average values was observed. Slight changes in blood calcium are usually associated with non-pathological or transient conditions [17].

Analysing joint fluid the amount of type B synoviocytes in OA affected joint fluid 6 months after stem cell therapy decreased in 33.3 % of dogs, increased by 11.1 %, but remained unchanged in 55.6 %. In 22.2 % of dogs the amount of fibrin in the OA elbow joint fluid decreased after treatment, but 77.8 % of the dogs remained unchanged. In 88.9 % of dogs protein in synovium remained unchanged but 22.2 % decreased. The amount of neutrophils in the joint fluid after stem cell therapy decreased in 44.4 % of dogs, increased in 11.1 %, and remained unchanged in 44.4 %. Comparing the amount of erythrocytes in the OA affected elbow joint fluid before and six months after stem cell therapy, it decreased in 44.4 % of dogs, increased in 33.3 % of dogs, and remained unchanged in 22.2 %. Six months after stem cell therapy, lymphocyte counts decreased in 33.3 % of dogs and remained unchanged in 66.6 %. Macrophage count after therapy in 11.1 % dogs increased but in 88.9 % remained unchanged. There were no statistically significant changes in synovial fluid analysis comparing data before and 6 months after therapy ($p > 0.05$) (Table VIII).

TABLE VIII: SYNOVIAL FLUID ANALYSIS BEFORE AND 6 MONTHS AFTER THERAPY

	Before therapy	6 months after therapy, with significance level
Type B synoviocytes ± SD	1.44 ± 1.01	1.00 ± 0.71 p = 0.257
Fibrin ± SD	0.56 ± 1.13	0.11 ± 0.33 p = 0.180
Protein± SD	0.89 ± 1.45	0.44 ± 1.33 p = 0.157
Neutrophils ± SD	1.00 ± 0.50	0.67 ± 0.50 p = 0.180
Erythrocytes ± SD	1.44 ± 1.01	1.67 ± 1.50 p = 0.796
Lymphocytes ± SD	0.67 ± 0.29	0.33 ± 0.87 p = 0.083
Macrophages ± SD	0.0 ± 0.0	0.11 ± 0.11 p = 0.317

From owners questionnaires after stem cell therapy 77.8 % of dogs were less lame, but 22.2 % of dogs lameness remained the same. Six months after therapy 55.6 % of dogs lameness was not observed, 22.2 % was observed sometimes, and for 22.2 % lameness was observed constantly a lightly (Table IX). At end of study lameness statistically significantly decreased ($p = 0.014$) (Fig. 7) where lameness score points: 1 - is not lame, 2 - sometimes lame, 3 - constantly a little bit lame, 4 - constantly lame, 5 - not walking on affected leg.

According to the owners questionnaires, where points: 1 - without pain, 2 - sometimes minimal to mild pain, 3 - constantly minimal to mild pain, 4 - constantly moderate pain, 5 - constantly severe pain, 100 % of the dogs were pain-free 6 months after treatment (Table X). Compared by months, the pain decreased statistically significantly ($p < 0.01$) (Fig. 8).

According to the animal owners assessment in points: 1 - not stiff, 2 - sometimes minimal to mild stiff, 3 - constantly minimal to mild stiff, 4 - constantly moderate stiff, 5 - severe stiff, do not want to walk, stiffness was decreased in 66.7% of dogs 6 months after treatment. At the end of the study, stiffness was not observed in 55.6%, for 22.2% of dogs sometimes mild stiffness was observed in and constantly mild stiffness was observed 22.2% of dogs (Table XI). Compared by month, stiffness decreased statistically significant ($p = 0.01$) (Fig. 9).

According to data from animal owners questionnaires where the points: 1 – getting worse, 2 – no changes, 3 – mild improvement, 4 – good improvement, 5 – excellent improvement, 88.9% of the dogs showed an improvement in their quality of life in the sixth month after stem cell therapy. In the study, excellent improvement was seen in 11.1% of dogs, good improvement in 44.4%, mild improvement in 33.3%, and no improvement in 11.1% of dogs (Table XII). Compared data before and six months after stem cell therapy, the quality of life improved statistically significantly ($p = 0.01$) (Fig. 10).

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TABLE VI: HAEMATOLOGICAL ANALYSIS BEFORE AND 6 MONTHS AFTER STEM CELL THERAPY

	Units	Before therapy ± SD	6months after therapy ± SD	Reference values
WBC	10^3 µL	11.46 ± 2.22	10.55 ± 1.78	6 - 12
RBC	10^6 µL	6.56 ± 0.52	6.81 ± 0.49	5.5 - 8.5
PCV	%	46.41 ± 5.02	47.77 ± 3.46	44 - 52
HGB	g/dL	16.68 ± 1.13	17.26 ± 0.93	15 - 19
MCV	fL	70.64 ± 3.99	70.67 ± 3.69	60.0 - 77.0
MCH	pg	25.46 ± 0.81	25.47 ± 1.07	19.5 - 24.5
MCHC	g/dL	36.09 ± 1.69	36.46 ± 1.73	32.0 - 36.0
RDW%	%	13.41 ± 0.49	13.64 ± 0.59	12.0 - 17.5
RDW a	fL	13.41 ± 0.49	54.97 ± 4.43	35.0 - 65.0
PLT	10^3 µL	277.78 ± 93.85	276.94 ± 103.78	166 - 575
MPV	fL	8.5 ± 0.48	8.22 ± 0.97	5.5 - 10.5

Leukocyte formula	%	10^3 µL	%	10^3 µL	%	10^3 µL
Metamyelocytes	0	0	0	0	0	0
Banded neutrophils	1.25 ± 0.96	0.15 ± 0.11	0.44 ± 0.88	0.05 ± 0.10	0 - 3	0 - 0,3
Neutrophils	69.67 ± 10.23	7.99 ± 2.04	69.0 ± 9.99	7.54 ± 1.89	60 - 70	3.0 - 11.5
Eosinophils	6.11 ± 5.39	0.69 ± 0.60	6.22 ± 5.07	0.77 0.56	2. - 10	0.1 - 1.25
Basophils	0	0	0	0	0	0
Monocytes	3.67 ± 2.5	0.44 ± 0.35	2.67 ± 2.67	0.29 ± 0.31	3 - 10	0.15 - 1.35
Lymphocytes	21.11 ± 9.94	2.39 ± 1.15	20.67 ± 8.63	2.13 ± 0.89	12 - 30	1.0 - 4.8

Abbreviations: WBC - white blood cell, RBC - red blood cell, PCV - haematocrit, HGB - haemoglobin, MCV - mean corpuscular volume, MCH - mean corpuscular haemoglobin, MCHC - mean corpuscular haemoglobin concentration, RDW% - percentage red blood cell volume distribution width, RDW a - absolute red blood cell volume distribution width, PLT - platelet, MPV - mean platelet volume.

TABLE VII: BLOOD BIOCHEMICAL ANALYSIS BEFORE AND 6 MONTHS AFTER STEM CELL THERAPY

	Units	Before therapy ± SD	6 months after therapy ± SD	Reference values
BUN	mmol/L	6.38 ± 0.91	6.37 ± 1.11	3.6 - 10.0
CREA	µmol/L	83.77 ± 19.99	79.39 ± 19.14	44 - 133
TBIL	µmol/L	0.16 ± 0.39	0.19 ± 0.41	0.0 - 10.2
ALAT	IU/L	44.23 ± 8.57	46.22 ± 8.3	21.0 - 102.0
ASAT	IU/L	41.43 ± 12.65	41.06 ± 12.39	23.0 - 66.0
ALP	IU/L	43.15 ± 37.21	51.25 ± 24.71	0.0 - 156.0
AMY	IU/L	725.15 ± 231.56	740.64 ± 206.25	350 - 1650
TP	g/L	73.53 ± 8.09	75.58 ± 6.71	53.0 - 76.0
ALB	g/L	37.03 ± 2.93	37.85 ± 1.89	25.8 - 47.0
P	mmol/L	2.15 ± 0.99	1.90 ± 0.90	0.5 - 2.6
Ca	mmol/L	2.2 ± 0.24	2.32 ± 0.22	2.32 - 2.82
Na	mmol/L	147.8 ± 3.56	146.83 ± 3.6	141.1 - 152.3
K	mmol/L	4.96 ± 0.91	4.75 ± 0.7	3.9 - 5.65

Abbreviations: BUN - blood urea nitrogen, CREA – creatinine, TBIL - total bilirubin, ALAT - alanine aminotransferase, ASAT - aspartate aminotransferase, ALP - alkaline phosphatase, AMY – amylase, TP - total protein, ALB – albumin, P – phosphorus, Ca – calcium, Na – sodium, K – potassium.

TABLE IX: LAMENESS EVALUATION BY MONTHS

	Before therapy	1 month after therapy	2 months after therapy	3 months after therapy	4 months after therapy	5 months after therapy	6 months after therapy
Lameness ± SD	2,67 ± 0,71	2,00 ± 0,5	1,78 ± 0,67	1,89 ± 0,78	1,78 ± 0,83	1,67 ± 0,87	1,67 ± 0,87

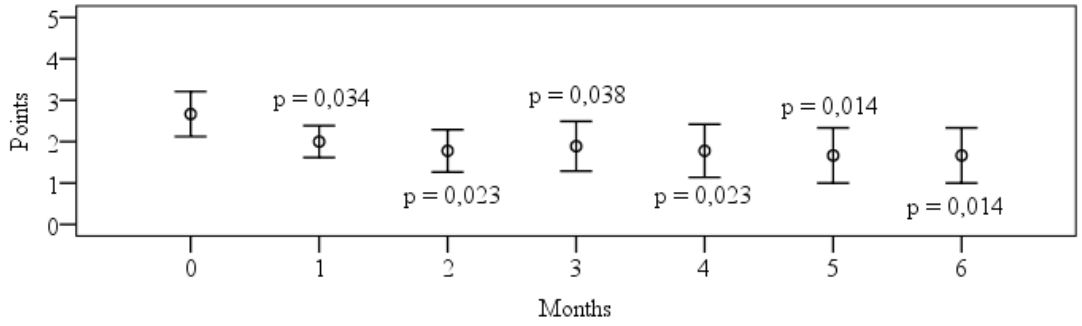

Fig. 7. Average values of lameness in points by months.

TABLE X: PAIN EVALUATION BY MONTHS

	Before therapy	1 month after therapy	2 months after therapy	3 months after therapy	4 months after therapy	5 months after therapy	6 months after therapy
Pain ± SD	2,11 ± 0,33	1,33 ± 0,50	1,11 ± 0,33	1,11 ± 0,33	1,11 ± 0,33	1,22 ± 0,44	1,22 ± 0,44

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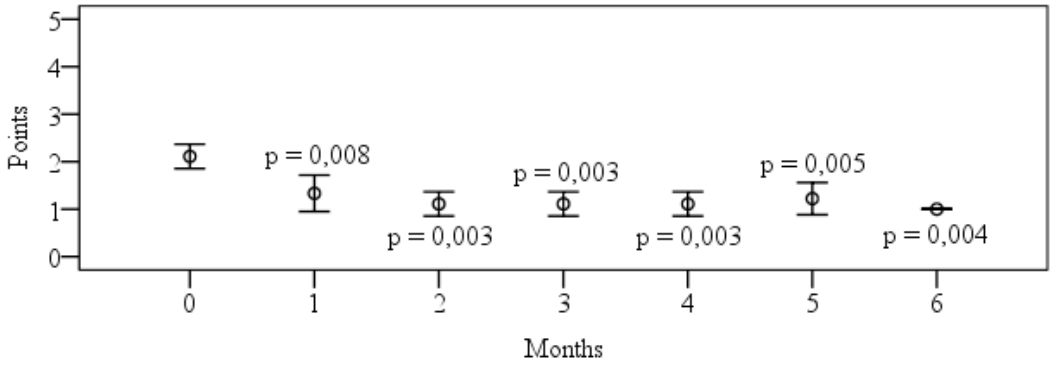

Fig. 8. Average values of pain in points by months.

TABLE XI: STIFFNESS EVALUATION BY MONTHS.

	Before therapy	1 month after therapy	2 months after therapy	3 months after therapy	4 months after therapy	5 months after therapy	6 months after therapy
Stiffness ± SD	2,56 ± 0,88	1,78 ± 0,83	1,67 ± 0,87	1,78 ± 0,83	2,00 ± 1,12	1,78 ± 0,97	1,67 ± 0,87

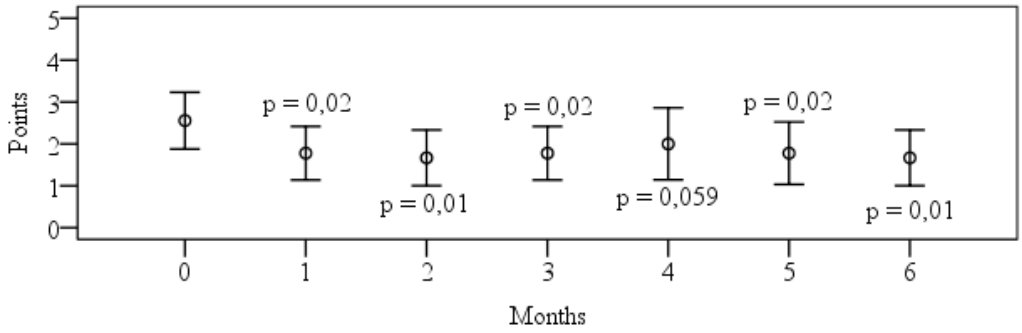

Fig. 9. Average values of stiffness in points by months.

TABLE XII: QUALITY OF LIFE EVALUATION BY MONTHS

	Before therapy	1 month after therapy	2 months after therapy	3 months after therapy	4 months after therapy	5 months after therapy	6 months after therapy
Quality of life ± SD		3,33 ± 1,00	3,33 ± 0,71	3,33 ± 0,87	3,33 ± 1,00	3,56 ± 0,88	3,56 ± 0,88

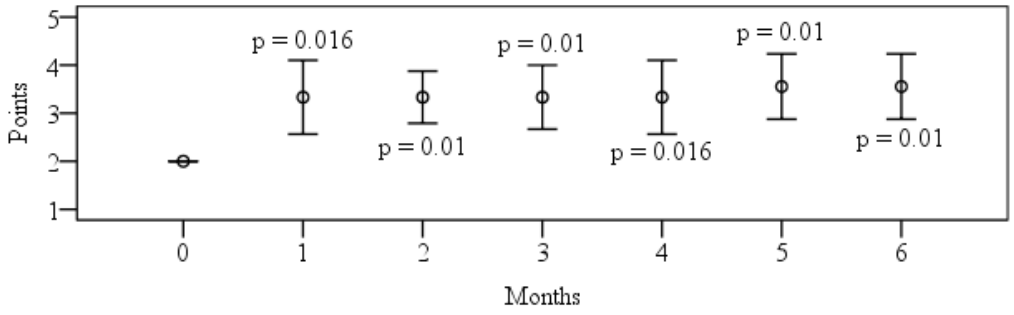

Fig. 10. Average values of quality of life in points by months.

IV. Discussion

The aim of this study was to evaluate whether an intra - articular MSC injection has a long - term beneficial effect on weight bearing, joint range of motion, radiologically observable OA signs, the composition of synovium, lameness and thus improves the quality of life and is safe to use.

The adipose-derived MSC therapy is a rapidly growing area of research, and it has been shown that stem cells have an affinity for damaged joint tissue [10]. Due to their immune evasive nature, MSCs release immunomodulatory factors which allow them to escape rejection mechanisms for sufficient time to exert their therapeutic action [12].

Intra-articular injection and stem cell product application in canine elbow joint is relatively simple manipulation. As mentioned in previous researches, due this study for none of dogs any adverse effects was not observed [13]. Animals tolerated the cell injection well, and there was no evidence of

local inflammation, immobilization, or unloading of the joint resulting from the cell treatment [18].

As indicated from previous studies stance analyser allow to detect differences on weight bearing overtime after treatment [14] and has been reported as sensitive for detecting lameness in dogs, with better results in large breed dogs [19]. From measurements on a stance analyser in this study weight bearing on OA affected leg increased but not statistically significant, it shows that pain in the joint was less. Chapman et al. [20] described MSCs intra-articular injection significantly reduced weight-bearing difference, which is a surrogate index of pain on loading. In this research stance analyser where in a relatively small room next to wall, at time of examination animals showed some anxiety, and tried to get off the scales, so it possibly could affect the results. It is possible that with some more adaptation time in the examination room and if the scales would be placed in centre of room dogs could be calmer and would be stand still.

One of the symptoms of OA patients is limited ROM, a reduction in the ability to move joints [21]. Goniometry is a simple and non-invasive method to quantitate the ROM of joints and has been used in canine orthopaedics to assess treatment efficacy. It is described that the results of goniometric measurements for sedated dogs and awake dogs did not show significant difference from results of radiographic measurements. [22]. For trial in this study there was tried to do goniometry for sedated dog with radiographic method, it was easier to perform but in this way animal and personal gets more x-rays and result was very similar as measured with plastic goniometer. In this study only for 33.3 % of dogs flexion angle decreased, but for 55.6% increased what means the joint was less foldable. Extension angle increased for 66.7 % of dogs but not significantly, what shows the joint can be stretched more and in total ROM increment was minimal from 97.22° ± 6.30° to 97.56° ± 4.21°.

Radiographic hallmarks of cartilage pathology include mainly joint space narrowing, subchondral sclerosis, subchondral cysts, osteophyte formation, and chronic inflammation of ligaments [23]. In this study osteophyte and STS was used as quantifiable parameters. Radiographic measures of osteophytosis are commonly used as components of the assessment criteria in elbow dysplasia (ED) screening schemes [24]. In this study osteophyte on the anconeal process was absent in 33.3 % of dogs, were compared to one other study absence of osteophytosis was detected in 28.8 % of clinically affected dogs [24]. Osteophyte decreased in mean size by 23.5 % but not statistical significantly. STS % decreased in 66.7 % of dogs in mean size by 7.88 %. In other studies STS was evidenced in ~96 %, 86.7 %, and 62 % of elbow joints diagnosed with medial coronoid process disease (MCD) [25] so evaluation of STS should be preferable for OA elbow joints with MCD as primary pathology. Further studies are needed to understand correlation between STS and severity of OA.

Osteoarthritis is a chronic inflammatory joint disease which changes in the synovial fluid [26]. In this study in joint fluid analysis type B synoviocytes, fibrin, protein, neutrophils, erythrocytes, lymphocytes, macrophages was detected where data was given and graded in points. All detected parameters, except macrophages, comparing before and 6 months after therapy decreased but not significantly. Macrophages not significantly increased.

Clinically, the major presenting signs of OA are lameness, stiffness, exercise intolerance, pain [27] and the quality of life of the affected animals is expected to decrease gradually [13].

Animal owner questionnaires gives information and data of their observations about the effectivity of treatment. For a better and more accurate assessment of the research, there should be included a placebo group. At end of the study from owners' assessments lameness, pain, and stiffness significantly decreased and quality of life significantly increased.

In conclusion, significant improvements in this study were observed only from the subjective comparisons of the owners with the initial state. Eight of nine owners were very satisfied. Other examinations showed improvements but not significant. Taking into account the results of the study overall suggest that allogeneic adipose-derived MSC treatment is beneficial for dogs suffering from OA without the risk of adverse effects on health. Two owners approximately 2 years after treatment reported that dogs become lame again and repeated stem cell intra-articular injection. There are no reports of other dogs.

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