Praca oryginalna *Original paper*

Ultrasound monitoring of the regenerative treatment of Biceps Tendonitis and Tenosynovitis in dogs by stem cells injections

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Received 06.06.2023 *Accepted 02.07.2023*

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Summary

Injuries and the overloading of the bicipital muscle tendon in dogs are a common cause of Bicipital Tendinitis and Tenosynovitis (BBT). The aim of the study was to evaluate the advantage of ultrasound monitoring during regenerative treatment of BBT together with treatment assessment by dog owners. 32 dogs of both sexes were used in the study. The dogs were treated with adipose-derived mesenchymal stem cells with platelet-rich plasma (ADMSC + PRP) administered twice in a dose of 0.5 ml (15.0×10^6 cells/ml). The ADMSC + PRP was injected into the tendon sheath of the biceps and 0.5 ml was injected into the brachial joint cavity. During the research, pain evaluation was performed according to the Canine Brief Pain Inventor – Penn CHART (CBPI), ultrasound scans were also performed. Some of the changes in ultrasound images are permanent and the recovery is gradual and incomplete. The research showed a correlation between the results of the ultrasound scans and the owners of the CBPI questionnaire, this was correlated with the effects of regenerative therapy. Studies have shown the regenerative effect of ADMSC + PRP on the tendon sheath and biceps tendon of the brachii after 4 months.

Keywords: enthesopathy, biceps brachii tendinopathy, USG, platelet-rich plasma, adipose-derived mesenchymal stem cells

Bicipital Tendinitis and Tenosynovitis (BBT) are a common cause of lameness of the front limbs in dogs (2). These problems are generally observed in medium-sized or large dogs, middle-aged or older, without any gender predisposition (4, 14). Clinical examination reveals lameness of varying severity, which may be exacerbated by physical exertion. In most cases, the cause of the disease is unknown. However, there are hypotheses which suggest that the overloading of the limb may be the main reason (5). Shoulder joint structure and the amortization function of the tendon during intensive work can cause BBT (18). Repeated overloading and/or injuries in the biceps tendon may lead to bleedings in the surrounding synovium, as well as swelling and trophic disturbances of the tendon. This, in turn, results in a reduced supply of oxygen to the tissue, which may promote the development of ischemic syndrome triggering the process of cartilage metaplasia and also the formation of calcifications of the tendon area (7). Increased inflammation results in the infiltration of inflammatory cells into the area of the tendon and synovium which can lead to villous hyperplasia of the synovium. Adhesions in between the tendon and the sheath may cause pain and the limited functioning of the limb. The disease process weakens the biomechanical strength of the tendon which can even lead to the rupture of the tendon (2, 25). Magnetic Resonance Imaging (MRI) is the gold standard concerning the diagnosis of BBT, although an ultrasound examination has proven to be accurate enough to identify the disease (3). BBT therapy constitutes a significant clinical problem due to the long-lasting and challenging nature of healing limb lameness. The standard treatment is based on the RICE principle (Rest, Ice, Compression, Elevation), the use of steroidal and non-steroidal anti-inflammatory

drugs and physiotherapy (10, 25). Unfortunately, 33% of dogs demonstrate a relapse of the disease, and recurrences were reported (5, 10, 17) in 55% of dogs treated with different surgical techniques. Another trend in the treatment of tendon diseases is the use of ortho-biological therapy using platelet-rich plasma (PRP) and adipose-derived mesenchymal stem cells (ADMSC) to reduce the catabolic and fibrosis response (5, 8, 28). The aim of the study was to demonstrate the value of using ultrasound to monitor changes during the regenerative treatment of BBT in comparison with the assessment of treatments by dog owners.

Material and methods

Between January 2017 and March 2020, 32 client-owned dogs of both sexes with unilateral forelimb lameness, aged 2.5 to 7 years, were chosen for the study: border collies (n – 10), boxers (n – 4), German Shepherds (n – 5), crossbreeds (n – 9), Australian Shepherds (n – 3) and an American Stafford terrier (n – 1). Informed written consent was obtained from the owners of the dogs and is included in the study. All dogs underwent tests before the treatment began; morphological blood tests, biochemical blood tests, imaging studies. The aim of these examinations was to eliminate animals affected by neoplastic diseases, infections, the effects of long-term treatment with anti-inflammatory drugs and acquired thrombocytopaenia being the most common ailment in dogs (12). The diagnosis was based on an orthopaedic examination, and a pain sensitivity examination during the bending and straightening of the shoulder joint and compression of the biceps tendon of the front limb at the point of intertubercularsulcus, as well as on the ultrasound results. A clinical and orthopaedic examination was performed on conscious and alert animals without sedation. Five times during the studies, in 0^{th}, 1^{st}, 2^{nd}, 3^{rd} and 4^{th} month, an ultrasound examination was performed on dogs sedated with medetomidine (Cepetor; CP-PharmaHandelsges. D) at a dose of 1 mg/kg i.m., butorphanol (Butomidor; Richter Pharma AG, A) at a dose of 1 mg/kg i.m. For the ultrasound examination, the dogs were laid in a lateral position, and then the examined limb was abducted and externally rotated for easier access to the examined area. Ultrasonography was performed using a Samsung WS80A 18 MHz line probe (LA4-18B). The imaging technology uses coded harmonic frequencies. An ultrasound examination of the biceps tendon was carried out from the *tuberculum supraglenoidales capulae* to the *sulcus intertubercularis*, both longitudinal and transversal images were collected (Fig. 1-3). During the research, in 0^{th}, 1^{st}, 2^{nd}, 3^{rd} and 4^{th} month, pain evaluation was performed according to the Canine Brief Pain Inventor – PennCHART (CBPI) scale. The dog owners answered 10 questions on the CBPI form, the answers were based on a 1 to 10 scale. On the CBPI scale, a score of „0 points" represented no lameness (no pain), and „10 points" lameness (severe pain) (20). The acquisition, isolation, and application of ADMSC were performed according to the protocol of Medivet Holdings International Pty Ltd. Adipose Stem Cell Procedure Kit Protocol V: 1.1 2010© (MediVet America. MediVet America In-Clinic Training – Manual., 2012, http://www.medivet.net.au). In all cases, the adipose tissue for stem cell isolation was obtained from the subcutaneous area of the scapula, on average this amounted to 50 ± 10 grams, depending on the patient's body weight and the level of obesity. According to the Medivet America protocol, photobiostimulation (FB) was performed, and PRP was used to activate ADMSC (9, 26). In the prepared PRP, the number of platelets was 6 to 8 times higher than in the peripheral blood. The quantification of blood platelets was performed using calcein. The samples were measured using a flow cytometer such as FACSCanto II, Becton-Dickinson. ADMSC/PRP in a dose of 0.5 ml (15.0×10^6 cells/ml) (13). An injection was made into the tendon of the biceps muscle under ultrasound control. The first dose was administrated in the attachment of *tuberculum supraglenoidale* are and the second one was administered 0.5 cm below the first one. An additional 0.5 ml was administered into the cavity of the shoulder joint. The maximum amount of injected biopreparation per administration did not exceed the amount of ADMSC obtained from 20 grams of adipose tissue. The procedure was performed each time under antibiotic cover, with using Cefovecin 8 mg/kg (Convenia, Phizer, UK) one day s.c. Unused stem cells were divided into 0.5 ml doses (15.0×10^6 cells/ml) each and stored at the boiling point of liquid nitrogen, i.e. minus 195.8°C. The second infusion was administered two months after the beginning of the treatment.

Excel Windows 2010 software and the statistical packet Statistica 10.0 (Stasoft) were used for database preparation and also for statistical calculations. The results were presented as arithmetic means and standard deviations. A student's t-test was used for a significance of differences calculation. The statistical significance was determined for $p < 0.05$. The relationship between the variables was evaluated using correlation coefficients (r) according to Pearson with an assumed value $p < 0.05$. The Guillford scale was used to assess the correlation strength between parameters.

Results and discussion

The dogs selected for the study were non-sterilised animals aged between 2 and 7 years (mean – 3.91, median – 3.75) and weighed from 15 to 31 kg (mean – 22.82, median – 22) with a BCS (Body Condition Score) ranging from 3 to 9 (mean – 7.85, median – 7.25). The lameness had lasted from 6 to 18 months (mean – 12.5 months, median – 13.35 months).

Achronic state of BBT was diagnosed in all 32 cases. The results of the ultrasound examinations, the change in the number of parameters examined during treatment and the results of the CBPI forms analysis are presented in the figures and tables below.

In many cases the improvement in the state of the patient was in fact the reason for the owner withdrawing from the control examinations and the completion of the treatment. This activity impeded the reliable and statistical development of the clinical trials (25). Without a doubt, the offer of inexpensive, non-invasive tests as a part of the ongoing monitoring process seems to be very appealing to owners. Ultrasound imaging remains a very useful and reliable diagnostic method

Tab. 1. Ultrasonographic changes identified during treatment

Time	Hypoechoic, thickened tendon	Excess synovial fluid within the tendon sheath	Hyperechoic areas within the tendon	Irregular intertubercular groove	Interstitial tear	Tendon sheath thickened	Total number of ultrasonographic changes
Month 0	32	32	15	32	12	25	148
Month 1	28	28	25	24	11	23	139
Month 2	21	17	23	20	8	18	107
Month 3	12	11	10	20	5	10	68
Month 4	3	5	4	11	3	3	29

for tendon diseases. Of course, magnetic resonance diagnostics remain the „gold standard", but ultrasound imaging has several advantages over MRI. US is faster, more economical and in most cases it may be performed without anaesthesia or sedation. The main disadvantage of ultrasound diagnosis remains the inability to assess the medial area of the shoulder joint (2). Standard BBT treatment consists of a conservative treatment with steroidal and non-steroidal anti-inflammatory drugs and rehabilitation (13, 22). The lack of any significant response to the standard treatment implies that other options should be considered, these include regenerative or orthobiological therapies (7, 10, 13). The studies of authors such as: Boswell et al., Canapp or Mishra et al., Moraes et al., present the effects of such treatments for tendinopathies and soft tissue injuries of the musculoskeletal system and encourage the selection of this type of therapy (1, 6, 19, 24). The ultrasonographic changes selected in the study were considered to be reference points to monitor the disease. During the study, the total number, quality and quantity of US changes were assessed (Tab. 1). ADMSC and PRP were applied at the beginning of the study (month 0) and in the second month of the study. The studies showed that the total number of ultrasound findings decreased after the administration of ADMSC and PRP from period to period. The inter-period number of ultrasonographic changes decreased between months 0 and 2 (after the first administration) and was 41 (Fig. 3). After the second administration, between

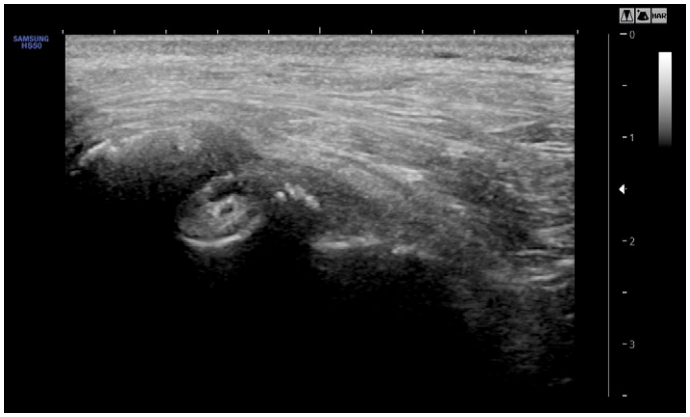

Fig. 1. German Shepherd, male, 4 years old. Transverse image of the biceps tendon. An interstitial tear is visible in the centre of the tendon. Samsung WS80A, 18 MHz linear probe (LA4-18B). Month 0 of therapy

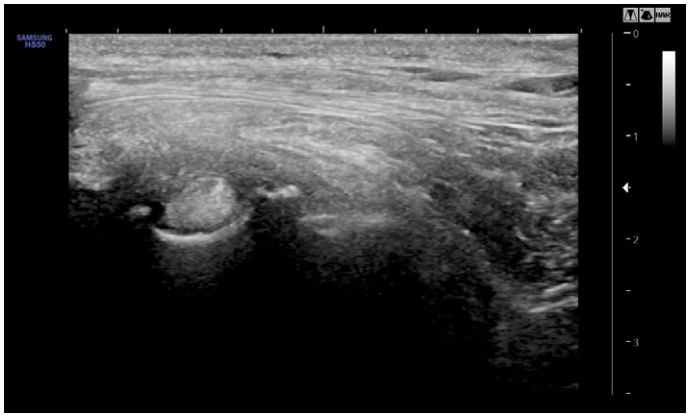

Fig. 2. German Shepherd, male, 4 years old. Transverse image of the biceps tendon. Hyperechogenic areas, post-inflammatory remodelling. 4th month of therapy. Samsung WS80A, 18 MHz linear probe (LA4-18B)

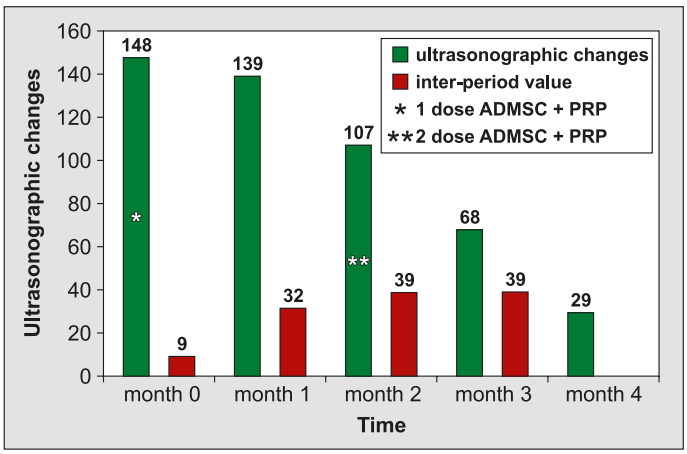

Fig. 3. Quantitative changes in the total number of ultrasound changes identified during treatment

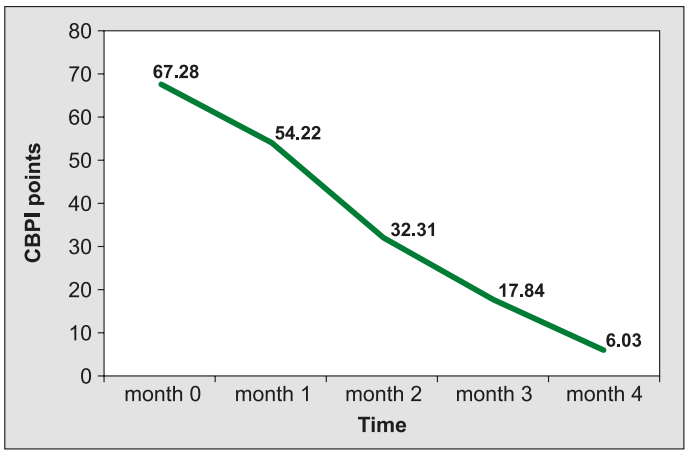

Fig. 4. Pain score, results in the CBPI scale in 0th, 1st, 2nd, 3rd and 4th month

months 2 and 4, it equalled 78 ($p < 0.05$) (Fig. 3). The average decline in the „total number of ultrasound changes" between periods was 29.75 (Fig. 3). These data show the dynamics of withdrawal and the direction of the quantitative trend. The study showed that the dynamics of the decrease in the number of ultrasonographic changes is more substantial after the second administration of ADMSC + PRP. This observation is consistent with the observations of other authors describing the effects of PRP and stem cells isolated from adipose tissue on the change in tendon echogenicity in supraspinatus tendinopathy in dogs (6, 15, 16, 22, 23, 30). During the period of the study, the Pearson correlation (according to the Guillford scale) was used to estimate the number of individual ultrasound findings and the CBPI scoring reported by the owners. The results of these observations are presented in our paper. The correlation coefficient (r) was positive in all cases and its value ranged from 0.646 to 0.998 (Tab. 2). In 8 cases out of 9, the coefficient (r) expressed a very high positive correlation (r = 0.998 to r = 0.939) (Tab. 2). Only in "hyperechoic areas" was the value of the correlation coefficient (r) 0.646, which represents a high positive correlation (Tab. 2). Our study has shown that in chronic cases of BBT (median – 13.35 mo.), the use of therapy using ADMSC + PRP requires further applications of the biopreparation to the tendon, which was consistent with the observations of other authors describing the results of orthobiological therapy in dogs (6, 15, 16, 22, 23, 30). A symptom called "irregular intertubercular groove" faded during the treatment, it was present in the biceps tendon of the shoulder muscle in all periods of the study. Its presence in the fourth month of the study in 11 cases may be the result of ongoing remodelling in this area. The pathology of the "hypoechoic tendon areas" (with an abnormal image of the tendon fibres) was evident in each period of the US study, they were particularly dominant at the beginning of the treatment. During the treatment, the images of these pathologies changed as they became normoechogenic images of the tendon-specific, filamentous. The number of ultrasound findings and their severity are associated with the strength of the inflammatory factor and the duration of the inflammation. Over the course of the ultrasound examination, a hypoechogenic to anechoic area around the biceps muscle tendon is often observed, which indicates an excess of fluid accumulated in the tendon sheath (29). According to some authors, the symptom of „excess fluid in the tendon sheath" is not BBT-specific (13).

Tab. 2. Pearson correlation coefficient values (r) between the average CBPI and the average of the ultrasound changes determined during the study

	SD	Average CBPI					r
		67.28	54.22	32.31	17.84	6.03	
Average US changes	total number of ultrasonographic changes	148	139	107	68	29	0.972
	hypoechoic, thickened tendon	32	28	21	12	3	0.980
	excess synovial fluid within the tendon sheath	32	28	17	11	5	0.998
	hyperechoic areas within the tendon	15	25	23	10	4	0.646
	irregular intertubercular groove	32	24	20	20	11	0.939
	interstitial tear	12	11	8	5	3	0.991
	tendon sheath thickened	25	23	18	10	3	0.968
		0 mo.	1 mo.	2 mo.	3 mo.	4 mo.	

The dynamics of the changes to this symptom in each period of the study are presented in our paper. The results show the possibility of the simultaneous coexistence of various ultrasonographic changes. This postulate is also included in the studies of other authors describing pathologies in the area of the shoulder and/or elbow joint which is not uncommon in dogs (13). The developing inflammatory reaction causes cellular infiltration into the tendon and synovium of the tendon sheath (11, 18). The aggravation of BBT leads to hyperechoic tissue aggregation and is the cause of the „thickened tendon sheath". The „thickened tendon sheath" as well as the symptom of „ thickened tendon" were noted in all periods of the study. The number of changes noted as „thickened tendon sheath" decreased from period to period however the „thickened tendon" was a symptom recorded at all periods. An analysis of CBPI questionnaire revealed that the occurrence of the „thickened tendon" symptom was more frequent and mainly reported in dogs undertaking physical activity despite their ongoing discomfort due to disease, this occurred as a result of a lack of proper care. In all likelihood, in such cases, the movement of the inflamed tendon inside the intertubercular groove caused pain which promoted further inflammation and intensified lameness (11). The failure of the owners to comply with the therapeutic recommendations meant that the dogs affected by BBT had worse results despite the treatment. The presence of an ultrasound symptom such as the „hyperechoic area" is described in the literature as disease progression towards tendon fibrosis (3, 10, 18). The study showed an initial increase (from the 0th month to the 1st month of the study) and then a decrease (from the 2nd month to the 4th month of the study) in the number of „hyperechoic area" symptoms. In considering the literature reports, the therapy results achieved should be considered significant and important. The „hypoechoic areas" are the result of a degradation in the filamentous tendon structure. The development of this symptom is the „interstitial rupture" which is visible through the use of ultrasound as a focal hypoechoic area with fibre deformation with or without destroying the tendon fibres (14). The study

shows that the change: "interstitial rupture", disappeared gradually throughout the study. However, by observing this symptom in „all ultrasonographic changes" one may notice a quantitative fluctuation as well as its significant contribution to the disease. This may indicate the importance of this type of tendon pathology and its resistance to ongoing regenerative therapy. Ultrasound monitoring showed the regenerative effect of ADMSC + PRP on the structure of the biceps tendon and tendon sheath over a four-month period. The study showed the retreat of ultrasonographic changes characteristic of BBT pathology. The proportion of normoechogenic images which are characteristic of the normal tendon structures increased over time. The quantitative dynamics of the changes may be observed in tables and figures. The effect of using ADMSC + PRP was a reduction in lameness and pain, as well as an improvement in limb function. The same observations are described in the works of Cook et al. and Lee and colleagues (11, 21). The ultrasound technique allowed for the tracking of the effects of regenerative treatment and the visualization of progressive changes in tendon morphology in real time, which is emphasised in the works of other authors (14, 23). Ultrasound monitoring of BBT treatment appears to be an effective method of monitoring the effects of therapy and showing the dynamics of the diminution of pathological changes. ADMSC + PRP is a method worth using in advanced BBT cases, especially when the standard treatment does not bring about the expected results. The results of the ultrasound studies were highly correlated with clinical observations, the satisfaction of the owners and the CBPI questionnaire results.

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