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CASE REPORT

Companion or pet animals

Rapid autologous point-of-care transplantation of the adipose-derived stromal vascular fraction in a dog with cubarthrosis

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Abstract

A 1-year-old shepherd dog was presented in the veterinary hospital due to left-sided cubarthrosis and persisting weight-bearing lameness of the left forelimb after a fragmented coronoid process in the left elbow joint had been removed at the age of 6 months. An autologous point-of-care transplantation of adipose tissue-derived regenerative cells was performed using ARC System (InGeneron, Houston, TX, USA). Pre- and postoperative investigations included orthopaedic and radiographic examinations, gait analyses as well as two owner questionnaires, Liverpool Osteoarthritis in Dogs and Canine Brief Pain Inventory. After 1, 2, 3, 6 and 12 months of treatment, the dog showed an improvement of peak vertical force and vertical impulse in the gait analyses as well as. The Canine Brief Pain Inventory and the Liverpool Osteoarthritis in Dogs revealed an improvement of the quality of life within all further control visits up to 12 months after the therapy.

BACKGROUND

In veterinary medicine, adipose tissue-derived regenerative cells (ADRC) already gained significant importance for the treatment of osteoarthrosis in horses and dogs.[1–5] The main focus of the therapeutic approach is in particular based on the immunomodulatory properties, which appear to lead to an anti-inflammatory and analgesic effect as well as an improvement of tissue healing and regeneration of articular cartilage.[6–17] Concentrating on the canine model, regenerative cells are usually isolated from fat or bone marrow and transplanted either autogenously or allogenously.[9,13] In terms of the clinical indication, recent veterinary studies already focused on the autologous transplantation of mesenchymal stem cells for the treatment of osteoarthrosis, particularly in the hip, the stifle and the elbow joints.[1–4]

Black et al. described the treatment of osteoarthrosis in the elbow joints of 14 dogs with the help of autologous, adipose-derived mesenchymal stem cells. All dogs had a history of a fragmented coronoid process (FCP) with surgical removal and/or osteochondritis dissecans. The tissue was taken from the abdominal, inguinal and falciform fat as well as fat from the thoracic wall. It was shipped to an external laboratory overnight for processing to inject the autologous isolated mesenchymal stem cells into the osteoarthritic joints thereafter. The effect of the therapy was evaluated by veterinarians, performing orthopaedic examinations, and by patient owner's questionnaires.

Vilar et al. performed objective gait analyses to access the improvement of a weight-bearing lameness in 10 dogs that had previously received mesenchymal stem cells in arthritic hip joints. While subjective pain assessment showed an improvement of lameness after 6 months, force platform data demonstrated that the lameness had reached initial values.[4]

All clinical studies that describe the use of regenerative cells in dogs suffering from osteoarthrosis cooperated with external laboratories where the cells must be shipped or sent to,[1–4] and partially even incubated for up to 2 weeks after having been extracted from the stromal vascular fraction (SVF).[3,4] Due to previous investigations on certain negative effects of transport and storage of equine regenerative cells on the viability and subsequent cell behaviour,[18] a rapid point-of-care procedure in preparing regenerative cells is described in the present case report. This new therapeutic approach is based on the extraction, isolation, processing and administration of the SVF without culturing the cells in an external laboratory.

Experimental studies have shown that the SVF contains regenerative cells, such as stem cells, endothelial cells,

fibroblasts, blood cells and their progenitor cells.[19–21] Furthermore, it has been shown in various experimental studies that the SVF provides a good molecular and cellular microenvironment for the contained regenerative cells.[16,17,22] The use of the ARC System (InGeneron, Houston, TX, USA), with its fast and effective procedure, represents an excellent point-of-care treatment in human medicine.[16,17] This is the first case report of in-house use of the ARC System to perform an autologous transplantation of the SVF in a dog with cubarthrosis. Objective force plate gait analysis was included to evaluate the objective status of lameness.

LEARNING POINTS/TAKE-HOME MESSAGES

- A rapid in-house procedure to isolate the stromal vascular fraction represents a possible tool to perform a fast autologous transplantation in joints of dogs suffering from mild osteoarthrosis and lameness.
- Using of orthopaedic investigation, gait analysis and owner's questionnaires, the lameness of a young dog with removed fragmented processus coronoideus and osteoarthritis improved after the autologous transplantation of stromal vascular fraction in the affected joint, which is to be considered a success.
- The causality between the injection of the stromal vascular fraction and the improvement in lameness of the patient could not be proven and must remain open.

CASE PRESENTATION

A 1-year-old white shepherd was presented in the veterinary hospital due to left-sided cubarthrosis and persisting weight-bearing lameness of the left forelimb. At the age of 6 months, arthroscopy of the left elbow joint has been performed in which a fragmented coronoid process (FCP) had been removed by an experienced surgeon. The treatment led to a positive short-term effect, but 4 months later, the lameness recurred and was permanently present. According to the patient's owner, the administration of non-steroid anti-inflammatory drugs subjectively led to a slight short-term improvement of lameness even though it persisted. Other causes for the lameness, such as infection or other orthopaedic problems, have been preliminarily ruled out.

INVESTIGATIONS

Clinical and orthopaedic examination

The dog was presented in a bright, alert and active condition. Vital parameters were within the norm. In the orthopaedic examination, the dog showed a clear weight-bearing lameness (grade 2/4). The left elbow joint was painful during extension and flexion, and increased joint filling was not evident. The examination of the right elbow and all other joints of the left and right limbs revealed no abnormal findings.

Radiography

Based on the radiographs of both elbows in two planes, left-sided cubarthrosis grade 1, following the International Elbow Working Group (IEWG), was diagnosed. The radiographs of the shoulders showed no abnormal findings (Figure 1).

Gait analysis

For the objective evaluation of lameness, gait analysis was performed using a treadmill with four integrated modified, piezoelectric force-measuring plates in a gang lab equipped with light-emitting diode light and high-speed infrared cameras. The investigation was analysed with the help of the software Vicon Nexus 2.7.1 (Vicon Motion System, Oxford, UK), QuadruPedLocomotion (Internal Software), Excel and SPSS (IMB SPSS Statistics 21.0/22.0). In all investigations, the speed of the treadmill was 1.2 m/s at walk and 2 m/s at trot. At least 20 steps per limb and examination were collected. Peak vertical force (PVF) and vertical impulse (VI) were measured. Symmetry indices of PVF and VI (SIPVF, SIVI) and the weight distribution were also calculated (Robinson et al.). To evaluate the kinematics, the range of motion (ROM) was determined.

Owner's survey

The patient's owner evaluated the quality of life of the dog with the help of two questionnaires, Canine Brief Pain Inventory (CBPI) and Liverpool Osteoarthrosis in Dogs (LOAD), preoperatively as well as 10 days and 1, 2, 3, 6 and 12 months postoperatively.

TREATMENT

The fur was clipped over the right cephalic vein, cleaned with water and disinfected before a vein catheter 20 G was placed. The bloodwork, including a large blood count and a serum profile before anaesthesia, was within normal limits. The anaesthetic protocol included benzodiazepine (diazepam 0.5 mg/kg) for the preanaesthetic period, propofol (2–4 mg/kg) for induction, followed by endotracheal intubation and maintenance with isoflurane (1.2–1.4 volume % in the expiration air) and oxygen. Methadone (comfortan 0.2 mg/kg) was given perioperatively for pain management, followed by the opioid buprenorphine (Buprenodale 0.02 mg/kg) directly after surgery. During anaesthesia, the patient received electrolytic infusion and vital parameters were monitored with the help of pulse oximetry, electrocardiography, blood pressure measurement and thermometry. The surgical field around the umbilicus was routinely clipped and disinfected. With a short incision in the linea alba (3 cm), the abdominal cavity was opened within the scope of laparotomy. Ten grams of falciform fat was removed, following a routine step-by-step wound closure. The whole surgical procedure lasted

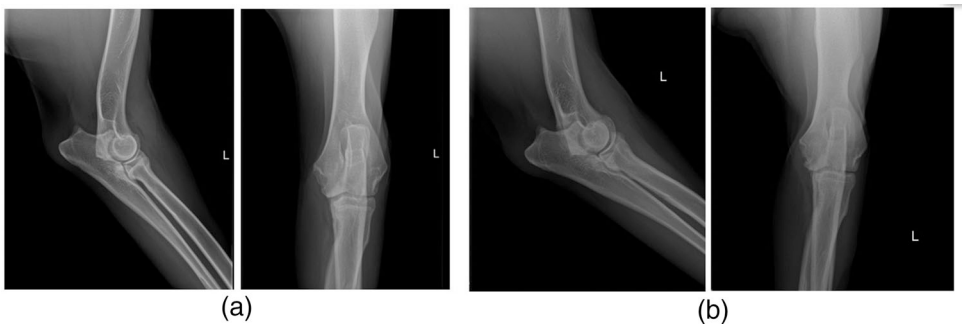

FIGURE 1 Laterolateral (left) and dorsoventral (right) radiographs of the affected joint before treatment (a) and 12 months after treatment. (a) Situation after coronoidectomy, subtrochlear sclerosis, mild bony proliferation cranial to radial head and on processus anconeus (grade 1). (b) Situation 12 months after treatment. Mild arthrosis (grade 1) still visible

approximately 10 minutes. The fat removed was collected in a sterile tube.

Using a point-of-care ARC System (InGeneron, Houston, TX, USA), ADRCs, the so-called stromal vascular fraction, were isolated from the fat removed. After thorough mincing of the fat removed, Ringer's lactate and the enzyme maltase were added. After several steps, including centrifugation, filtration and washing, the cells were ready for application within approximately 1.5 hours. All work steps were performed under sterile conditions.

During a short sedation of the patient using propofol, the left elbow joint of the patient was clipped, cleaned and disinfected. After aspiration of synovia, which was preserved using a cannula 20-G needle and a 5-ml syringe, the regenerative cell suspension of 1.5 ml was injected into the joint under sterile conditions. A small part of the suspension has been used for a live/dead cell count coloured with trypan blue and Cyto-13.

For the following 7 days after surgery, the owner administered an NSAID (carprofen 4 mg/kg once daily). After 10 days, the sutures of the surgery wound on the abdomen were removed.

OUTCOME AND FOLLOW-UP

The live/dead cell count results revealed that the number of injected cells was 6.97×10^6, with a viability of 65.17%.

Ten days after the procedure, clinical, orthopaedic examination and owner questionnaires were repeated. Follow-up examinations, including gait analysis, were performed 1, 2, 3 and 6 months after injection of the regenerative cells. In none of the clinical re-examinations the dog subjectively showed any lameness in walk or trot. Radiographs of the left elbow joint in two planes were repeated after 3 and 6 months confirming no progression of the cubarthrosis.

Concerning the gait analysis, PVF and VI of the affected limb were increased in all re-examinations for walking and trotting (Table 1). Only in the 6-month control, the animal refused to trot. Furthermore, SIPVF and SIVI were decreased in all re-examinations in comparison to the values before the surgery. Regarding SIPVF and SIVI, the dog was free of lameness in all re-examinations after surgery. Weight-bearing of the affected front limb increased after injection of the regenerative cells and remained above the preoperatively measured values (Figure 2).

The ROM of the elbows of both front limbs remained constant within all examinations (affected limb: minimum 42.3°, maximum 45.1°, non-affected limb: minimum 47.5°, maximum 54.0°). The mean difference between the ROM of the affected and the non-affected elbow was 8.5° ± 2.1°.

After treatment, the weight distribution of the patient during walking reflected an increase in weight load of the ipsilateral (treated) forelimb and a decrease of weight load of the contralateral forelimb (Figure 2). Focusing on the hindlimbs, the weight load decreased within all further control sessions. An improvement of the scores in the two owner questionnaires was detected in all follow-up visits (Table 2).

After a year, the owner was contacted by phone and he confirmed that the animal still did not show any lameness.

DISCUSSION

To our knowledge, this is the first case report that describes the in-house use of an ARC System for autologous transplantation of the SVF in a dog suffering from cubarthrosis due to a fragmented processus coronoideus, which was surgically removed before. In the present case, no causality between the injection of the SVF and the improvement in lameness of the patient could be proven. Nonetheless, the positive results correspond to those of the studies of Black et al. (2007, 2008), in which tissue processing was carried out in external laboratories, and the improvement of lameness after the therapy in dogs with cubarthrosis was investigated using orthopaedic examinations and owner questionnaires, even though a gait analysis was not performed.

The concordance of gait analysis and numeric subjective scoring scales was investigated by Vilar et al. to access the improvement of a weight-bearing lameness in 10 dogs that had previously received mesenchymal stem cells in arthritic hip joints. Subcutaneous fat tissue from the inguinal region of the dogs was used and cultured in an external laboratory. Mesenchymal stem cells were finally isolated and injected into the affected joints of the dogs. Even though subjective pain assessment showed improvement after 6 months, force plate data demonstrated that these animals had returned to the initial status of lameness. In contrast, the previous case demonstrated not only a short-term but also long-term therapeutic success. Whether the therapeutic success can be attributed to the regenerative character of the cells[16,17] could not be confirmed with certainty. As the lameness improved after the therapy,

TABLE 1 Peak vertical force (PVF), vertical impulse (VI) and symmetry indices (SIPVF, SIVI) of the affected front limb

Walking	Preoperatively	1 Month	2 Months	3 Months	6 Months	12 Months
PVF	46.6	56.9	56.1	57.1	63.2	61.5
VI	15.7	17.7	17.5	20.2	21.7	20.3
SD	71.5	68.0	67.0	72.0	70.0	69.5
SIPVF	13.0	5.0	2.9	6.6	4.9	3.83
SIVI	17.9	7.5	2.4	5.7	5.7	9.94
Trotting	**Preoperatively**	**1 Month**	**2 Months**	**3 Months**	**6 Months**	**12 Months**
PVF	59.2	78.5	79.4	79.9	–	92.8
VI	13.5	15.4	16.8	15.4	–	19.4
SD	60.0	58.5	62.0	59.0	–	59.0
SIPVF	31.3	12.5	5.9	8.3	–	0.11
SIVI	34.5	14.3	4.2	14.5	–	8.07

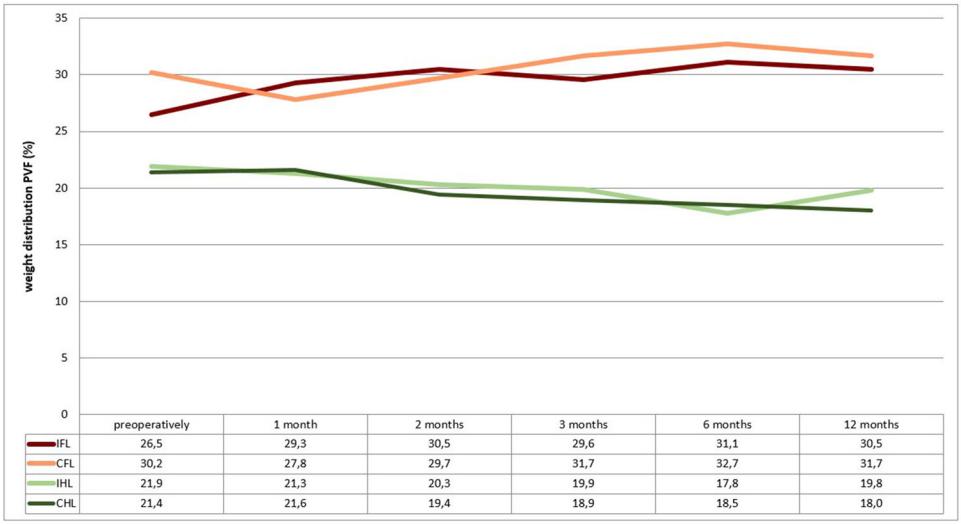

FIGURE 2 Weight distribution of peak vertical force during walking (IFL = ipsilateral [treated] forelimb, CFL = contralateral forelimb, IHL = ipsilateral hindlimb, CHL = contralateral hindlimb)

TABLE 2 Owner questionnaires Liverpool Osteoarthritis in Dogs (LOAD) and Canine Brief Pain Inventory (CBPI)

	Preoperatively	1 Month	2 Months	3 Months	6 Months	12 Months
LOAD	1.38	0.61	0.61	0.76	0.23	0
CBPI	4.8	2.5	2	1	1.6	1

the authors suspect that the success can be attributed to the injection even if this cannot be proven.

Focusing on the kinetic and kinematic results, the ROM reveals mechanical factors that determine extension and flexion of the joint.[23] While PVF and the VI of the affected limb improved and the ROM stayed stable within the follow-up checks of the present case, the improvement of lameness seems to be due to the analgesic effect and not due to the mechanical properties of the joint.

In dogs suffering from a weight-bearing lameness, a typical mechanism for compensation is characterised by a decrease in weight load on the ipsilateral limb and an increase in weight load of the contralateral limb. In addition, lameness of the forelimbs compensatory decrease the weight load of the hindlimbs and vice versa.[24,25] These mechanisms correspond to the current findings and the dog of the present case. As the compensated weight distribution of the patient was reversed during the follow-up sessions, a constant decrease of weight-bearing lameness could be seen.

In view of the surgical procedure, Vilar et al. isolated mesenchymal stem cells from subcutaneous fat of the inguinal region and Black et al. isolated stem cells from the abdominal, inguinal and falciform fat of the thoracic wall region, with good clinical results. For the present patient, the transplanted cells were isolated from falciform fat. Sullivan et al. attributed the extraction of mesenchymal stem cells out of the falciform fat to be a simple and successful method in dogs.

The number of injected cells in the present dog was 6.97×10^6. In accordance with the live/dead cell count, the viability

of the cells was 65.17%. Black et al. reported numbers of viable cells between 3 and 5 × 10^6 from 15 g of fat. This result is comparable to our yield of 4.5 × 10^6 cells, even though the exact number of injected mesenchymal stem cells is not given. In contrast, Vilar et al. counted 15 × 10^6 adipose mesenchymal stem cells after 2 weeks of cultivation, hence far more than in the current case, but difficult to compare due to another laboratory technique. A comprehensive characterisation of the injected cells has not been performed. This can be justified by the fact that it was a point-of-care treatment, which should be carried out as quickly as possible and without reducing the cell count.

To keep the investigations as objective as possible, the lameness of the dog was investigated multimodally, with the above-mentioned validated owner assessments as well as the gait analyses. Both the LOAD and the CBPI had been developed and validated to assess the quality of life, pain and lameness in dogs[26,27]; the results of both questionnaires in the current case corresponded to one another.

To rule out the possibility that the weight-bearing lameness of the patient was influenced by other joint defects in the present case, a complete orthopaedic examination of all other joints of the patient was performed as well as radiographs of the joints of both forelimbs were evaluated. Before the gait analyses, the patient did not receive any analgesia or corticosteroids to exclude any effects of medications on the lameness of the dog. Black et al. also determined the degree of osteoarthritis in dogs using orthopaedic examination and radiographs.[1,2] Vilar et al. included a gait analysis in addition to the orthopaedic examination and radiographs in their evaluation of osteoarthritis in dogs.[4]

Before treatment, left-sided cubarthrosis of grade 1, following the IEWG, was evident on appropriate radiographs in two planes. During orthopaedic re-evaluation after 3 and 6 months, a progression of cubarthrosis in the affected joint was not evident in the radiographs. The radiographs must be viewed critically as the degree of osteoarthritis does not necessarily correlate with the degree of lameness.[28] Considering the radiograph finding in the current case, only mild signs of degenerative osteoarthrosis were visible (grade 1). In contrast, Black et al. also included dogs with osteoarthrosis of grade 2 or higher, recording a successful therapy. Previous studies have shown that osteoarthritis always progresses after the treatment of an FCP.[29] Therefore, the fact that osteoarthritis stagnated in the current case should be seen as a success.

Our patient underwent stem cell therapy at the age of 2 years. Compared to recent studies, Shah et al. who investigated the allotransplantation of stem cells in dogs, the age of the donor animals was less than 5 years[30] and Black et al. included dogs with age of up to 11 years, with clinical success. The age of the patient in the present case was therefore very young. To what extent older dogs react to such therapy remains unclear. In the present case, the cartilage of the treated joint was predominantly intact according to the previous arthroscopy.

For this reason, future large-scale, comprehensive studies seem to be reasonable to verify the promising effect of the described procedure, also with respect to the age of the patients and the degree of arthrosis. Possible follow-up studies could focus on the treatment of end-stage osteoarthritis.

AUTHOR CONTRIBUTIONS

All authors contributed to both, conducting the study and writing the manuscript.

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CONFLICTS OF INTEREST

The authors declare they have no conflicts of interest.

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ETHICS STATEMENT

All investigations and treatments were approved by the Ethics Committee of the Centre of the Faculty of Veterinary Medicine, Ludwig-Maximilians-University Munich (application 31-20-06-2014).

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