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Stromal vascular fraction cell therapy for osteoarthritis in elderly: Multicenter case-control study

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1. Introduction

Osteoarthritis (OA) is the most common type of degenerative joint disease often requiring joint replacement as a current available therapeutic option. A basis of OA is degeneration and chronic inflammation of the connective tissues of joints, including the cartilage due to a long-term damaging of chondroblasts, chondrocytes and extracellular matrix[1] caused by oxidative stress, inflammatory factors and mitochondrial dysfunction causing DNA damage.[2] Elderly people are the most vulnerable population as effects of ageing including degenerative changes and chronic inflammation of joints are more pronounced.

Standard OA therapy is limited to symptomatic treatments by nonsteroidal anti-inflammatory drugs (NSAIDs), steroids and joint injections of hyaluronic acid (HA). Side effects of NSAIDs are well known but less known are severe side effects of elderly who typically suffer from some level of dehydration due to a lack of feeling thirsty. Other commonly prescribed medications in elderly may also represent severe health risk in addition to dehydration and cumulative toxicity of NSAIDs, especially in long-term use.[3] Other conventional therapy of OA includes physical therapy and arthroscopic lavage. When symptomatic conventional treatment failed, artificial joint surgery is the final standard way of available therapy. Nevertheless, such surgical treatment carries a high risk of complications, including increased risk of infection, thromboembolism, myocardial infarction, stroke, morbidity and even mortality,[4] namely in frailty population of elderly.[5] In total joint arthroplasty (TJA) of the cartilage metal-on-metal implants or ceramic-on-metal

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implants are commonly used. Metal nanoparticles such as cobalt, chromium, nickel, vanadium, titanium or molybdenum are released from the metal implants, as the result of wearing and corrosion, and may disseminate throughout the body, penetrate cell membranes, binding to cellular proteins or enzymes, thus modulating cytokine expression and having serious health consequences. Those may contribute to alteration of nervous system functions including memory loss, behavioral changes, depression, Parkinson's disease, Azheimer's disease, hypothyroidism and cardiomyopathy.[6,7] Also chromosomal aberrations and DNA damage were described as the consequence of heavy metal poisoning released from metal prosthesis. Increased risk of cancer in patients with artificial joint was also well described and documented from large population registry in Sweden in long-term 30 years follow-up.[8] Mainly increased risk of leukemia and melanoma, breast carcinoma in women, and prostate carcinoma in men was documented.[8–10] This data demonstrates that artificial joint may represent very serious threat namely for elderly as chronic cumulative toxicity where long-term use of NSAIDs and artificial joint are the most common standard solution for patients suffering from joint pain and OA worldwide.[11]

The association of OA with obesity, hypertension, dyslipidemia, metabolic syndrome and hyperglycemia was also described.[12,13] Commonly prescribed medications of these diseases may have further dramatic negative impact on OA, especially in elderly population.[5,13] Due to chronic and cumulative toxicity of standard approaches to OA, novel safe and minimally invasive medical procedures are needed, namely in elderly where the risks associated with their frailty are even more pronounced.

One of the possibilities for innovative and minimally invasive treatment of OA relies on local application of autologous mesenchymal stromal/stem cells (MSCs) isolated from either bone marrow or adipose tissue.[14] MSCs from adipose tissue can be safely isolated in much higher quantities, are more genetically stable, and may be easily obtained from adipose tissue by standard liposuction as the part of stromal vascular fraction (SVF).[15] MSCs are able to differentiate into osteoblast-, chondrocyte-, and tenocyte-like cells in the case of musculoskeletal application.[16] Local joint clinical application using adipose tissue-derived stem cells has been documented in humans with OA since 2011.[17] Adipose SVF cells have strong homing effect within the loose connective tissue which is homologous to adipose tissue – another form of loose connective tissue.[18] Recently, we were able to demonstrate significant clinical effects in large group of 1128 patients with OA, especially in improved quality of life including significant reduction of pain, decrease in NSAIDs usage, and other parameters closely related to joint functions.[19] To our knowledge, this is the world largest case-control clinical study documenting clinical effects of SVF cells in patients with OA.

In this article we focus on elderly population from our large clinical study, people older than 80 years as they are the most frailty. They are seeking for safe, effective and minimally invasive solution for their OA in order to improve quality of life without possible side effects which are more pronounced in this age group. We also focused on long-term 36 months follow-up of this unique and vulnerable population which was not previously documented or described in such extent elsewhere.

2. Materials and methods

All materials and methods are in agreement and were described in detail in our previous study.[19] All patients were older than 80 years (80–94 years old). All patients provided informed consent. Certified orthopedic surgeons and/or traumatology surgeons recruited patients with OA as agreed with the following criteria: (1) chronic or degenerative joint OA grade 2–4 (Kellgren-Lawrence) of 1–4 large weight bearing joints (including hip and knee) and additionally 0–8 other joints (shoulder, elbow, wrist, hand, ankle and foot) causing significant functional disability detected by clinical examination, X-ray and/or magnetic resonance imaging (MRI); (2) failure of conservative therapy; (3) signed informed consent form. Exclusion criteria included: (1) active inflammatory disease; (2) severe cardiac, pulmonary or systemic disease; (3) active neoplasm treated with immunosuppressive agents (including chemotherapy, radiotherapy, steroids and other immunosuppressives) within the past 12 months; (4) steroids or platelet-rich plasma (PRP) within the past 4 weeks; (5) health condition (including allergy to local anesthetic drug) that does not enable to perform liposuction in a local anesthesia safely; (6) pregnancy or lactation; (7) TJA. Candidates for TJA were allowed to participate in SVF therapy. All patients underwent standard tumescent liposuction under the local anesthesia to obtain 20–200 ml of lipoaspirate. Lipoaspirate was processed by Cellthera Kit I (patented technology: EP No. 2792741) or Cellthera Kit II, Cellthera, s.r.o., Brno, Czech Republic in order to obtain at least 15 million nucleated SVF cells. All nucleated SVF cells were counted on Burker chamber (Glaswarenfabrik Karl Hecht GmbH & Co KG, Sondheim/Rhön, Germany) after staining by trypan blue (Sigma-Aldrich, St Louis, MO, USA). 1–5 ml aliquot of SVF cells resuspended in autologous plasma (Cellthera Kit I) or CT-WSH solution (Cellthera Kit II) were applied to 4 large joints (hip, knee) or up to 8 other joints (shoulder, elbow, wrist, hand, ankle, foot) intraarticularly or periarticularly to the synovial stromal loose connective tissue closely to the joint. If necessary, ultrasound or C-arm X-ray navigation of the needle was employed. SVF therapy was recorded and evaluated before therapy and after 1, 3, 6, 12, 24 and 36 months after the SVF treatment.

Clinical evaluation included medical history, degree of OA and joint pain, number of analgesic drugs taken weekly, joint stiffness, limping at walk and extent of joint movement. OA score was constructed on the basis of the modified Knee/Hip Osteoarthritis Outcome score (KOOS/HOOS; www.koos.nu) as the mean value of parameters A-E for each patient:

(A) Pain – patient evaluation (0 = no pain; 1 = minor not frequent pain; 2 = minor frequent pain; 3 = moderate pain; 4 = severe pain; 5 = unbearable pain continuously requiring analgesics)
(B) Painkillers per week – physician evaluation (0 = no painkillers; 1 = 1–7 pills/topical analgesic cream (TAC); 2 = 8–14 pills/TAC; 3 = 15–21 pills/TAC; 4 = 22–28 pills/TAC; 5 = 29 or more pills/TAC)
(C) Limping at walk – physician evaluation (0 = no limping; 1 = less frequent minor limping; 2 = frequent minor limping; 3 = moderate limping; 4 = severe limping; 5 = impossible to walk)
(D) Extent of joint movement – physician evaluation (0 = no limitation; 1 = limitation less than 20%; 2 = limitation 21–40%; 3 = limitation 41–60%; 4 = limitation 61–80%; 5 = limitation more than 80% - impossible to move)
(E) Joint stiffness – patient evaluation (0 = no stiffness; 1 = minor; 2 = moderate; 3 = serious; 4 = severe; 5 = impossible to walk)

The non-parametric statistical analysis of changes in Scores over the time (before, at 1, 3, 6, 12, 24, and 36 months) in each treatment group was tested by one-way repeated measures analysis of variance (ANOVA). The significance level 0.05 was used throughout. The data were analyzed using statistical software STATISTICA v.10 StatSoft, Inc.

3. Results

A total of 29 patients aged 80–94 years (average age 83.3 years, 4 of them older than 90 years) were treated by SVF cells isolated from adipose tissue. 31.1% of patients were males and 68.9% were females. 10.3% patients were diagnosed with grade 2, 48.3% with grade 3 and 41.4% with grade 4 of degenerative osteoarthritis monitored by clinical and X-ray examination. There were 24.1% patients with normal weight (BMI 18–24.9), 41.4% overweight patients (BMI 25–29.9) and 34.5% obese patients (BMI 30 or over). Adipose tissue was processed leading to at least 15 million nucleated SVF cells per joint applied. No complications associated with processing of adipose tissue or preparation of SVF cells was noticed. Local pain and minor swelling at the site of injection were observed for 24–48 h after the SVF application in 2 cases. No long-term side effects or complications after the procedure were noticed during 36 months follow-up period. Three patients have died during the follow-up period (21–33 months after their SVF therapy) due to aging and causes not related to SVF therapy.

Regarding clinical effects of SVF therapy, semiquantitative evaluation of pain as well as total amount of NSAIDs or analgesic drugs were significantly decreased from the first month after the SVF therapy and were significantly decreased further during the next 36 months in comparison to the clinical situation before SVF therapy (Fig. 1A and B). Similar results were obtained by evaluation of limping at walk, extent of joint movement and joint stiffness (data not shown). Complex Score evaluation of all 5 parameters (pain, number of analgesics/NSAIDs per week, limping at walk, joint stiffness, and extent of joint movement) including statistical analysis is summarized in Fig. 2. A significant and constant improvement in Score was observed for 36 months after SVF therapy compared to the status before SVF therapy. Score was constantly decreasing for 24 months. Last follow-up at 36 months reveals mild non-significant Score increase. Only 2 patients (6.9%) required total hip replacement during the analyzed period (21 and 24 months after SVF therapy), both with grade 4 OA. One of these

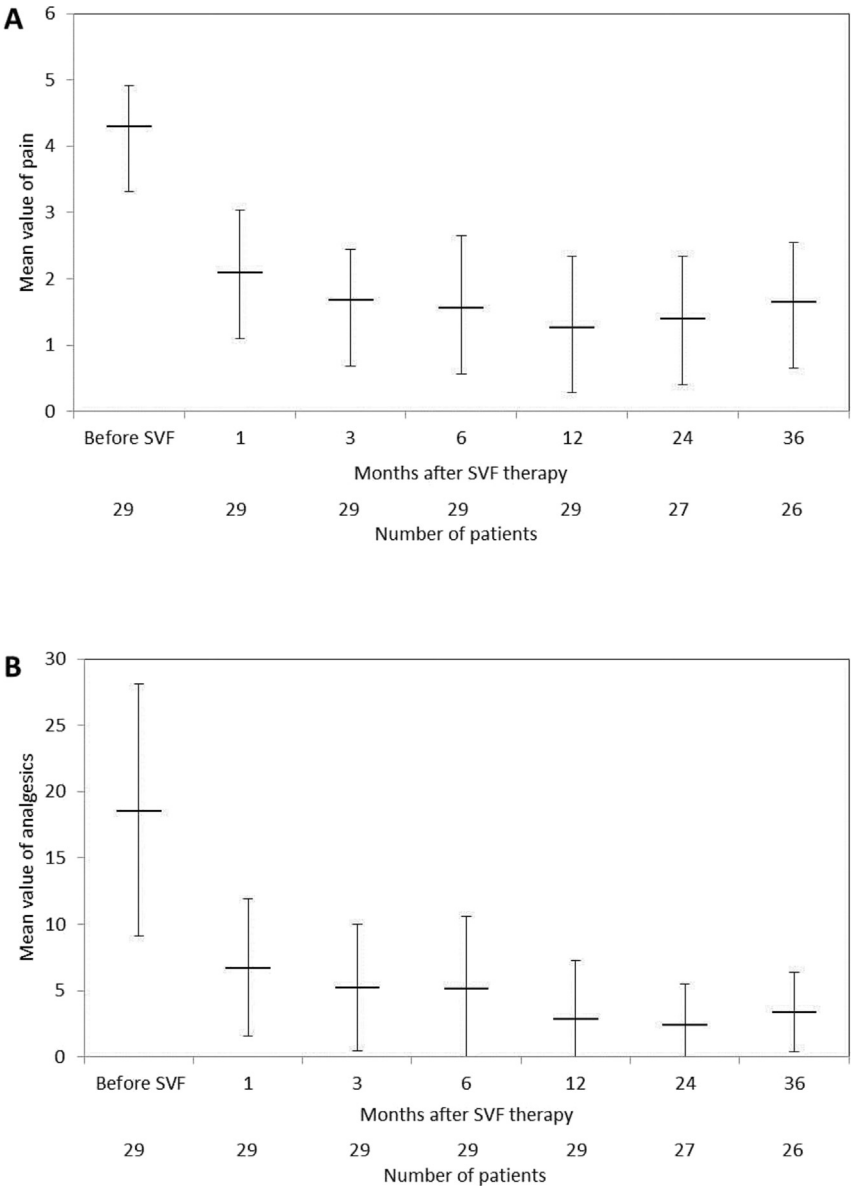

Fig. 1. Semiquantitative evaluation of pain and weekly analgesics consumption in OA patients during SVF therapy. The mean value (±1SD) of pain (A) and amount of weekly taken analgesic/NSAID drugs (B) significantly decreases after SVF therapy.

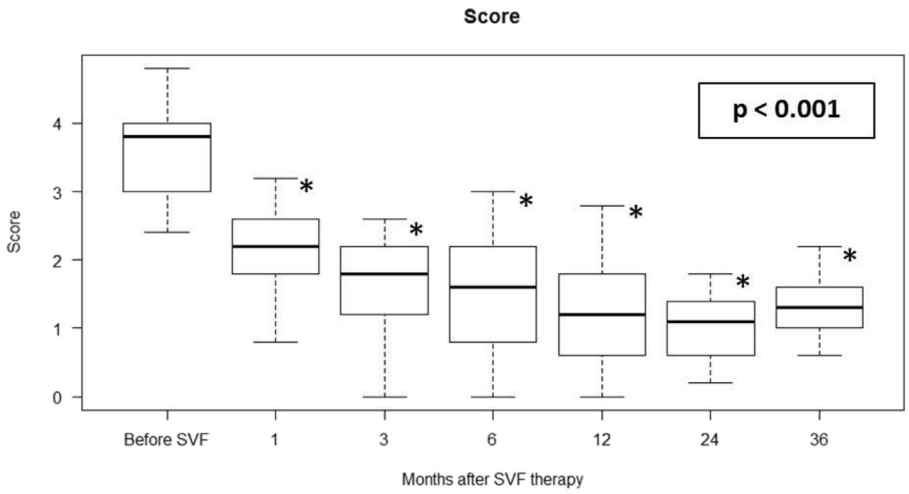

Fig. 2. Semiquantitative Score evaluation of 5 parameters: pain, number of analgesics/NSAIDs per week, limping at walk, joint stiffness and extent of joint movement. The significant improvement in Score (*) was observed after SVF therapy compared to status before SVF therapy ($p < 0.001$). Means ± SD [box] and ±1.96 SD [bars] are shown.

patients died 12 months after TJA.

In summary, SVF therapy represents important tool in regeneration of joints in elderly patients. Such a novel method can be provided very safely and gently during one surgical procedure with a respect to frailty of this patient population thus significantly improving their quality of life without the risk of serious side effects or complications.

4. Discussion

Osteoarthritis is one of the most frequent chronic diseases in middle-aged and elderly people which progressively lead to loss in quality of life. Strong correlation between increasing age and the prevalence of OA exists, so older people are affected more frequently. For the first time we had opportunity to describe results of autologous stem cell therapy using adipose tissue-derived SVF cells in elderly people over the age of 80 suffering from degenerative OA. Characteristic feature of elderly people is frailty often associated with chronic diseases and pain.[5] Chronic joint pain, the typical sign of degenerative OA negatively affects daily activities of patients and may lead to depression, decreased socialization and anxiety.[20] Analgesics and NSAIDs along with dehydration may lead to cumulative toxicity, hypersensitivity to NSAIDs and severe toxicity, especially in the gastrointestinal tract, cardiovascular system, kidneys, liver, lungs, joints etc.[21,22] Hafezi-Nejad et al.[23] described that long-term uses of analgesics leads to radiographic progression of knee OA and increases risk of future knee replacement. Joint replacement is quite frequently contraindicated in elderly due to their increased level of frailty.[5] Serious side effects or TJA are well documented including increased risk of infection, thromboembolism, myocardial infarction, stroke, other morbidity and mortality.[4] Thus, safer variant for increasing elderly population with OA is needed.

Various stem cells procedures have been used for OA treatment, for example bone marrow isolated MSCs,[24] adipose tissue-derived stem cells (ADSCs),[15,25] SVFs[19] or peripheral blood-derived stem cells.[26] SVFs and ADSCs have been clinically used in treatments of Parkinson's disease, spinal cord injury, amyotrophic lateral sclerosis, traumatic brain injury, dementia, stroke,[27] multiple sclerosis,[27,28] femoral head necrosis,[29,30] chronic myocardial ischemia,[31] acute respiratory distress syndrome,[32] Crohn's disease,[33] psoriasis,[34] Peyronie's disease[35] and others, including degenerative OA. MSCs are involved in immunomodulatory and repair processes in human body due to their own regenerative potential.[36] Repair effects of these cells are caused by production of multiple paracrine factors that downregulate proinflammatory cytokines and upregulate antiinflammatory cytokines leading to decrease of inflammation, immunomodulation, tissue repair, and improved proliferation and permeability of endothelial and epithelial cells.[37,38] In 2011, Pak[17] demonstrated for the first time the regeneration of cartilage tissue in patients with OA by using of autologous ADSCs in the form of SVFs with PRP and hyaluronic acid (HA). In other study PRP has been used for OA treatment alone. It was noticed that PRP reduced pain and improve knee function but without cartilage regeneration and just for a short period of time.[39] Intraarticular application of HA or PRP alone typically involves the series of intraarticular injections that may increase risk of side effects and infection, effects are rather short-lasting. On the other hand our results are based on a single injection of SVF cells which was not associated with any serious side effects and the clinical effects are long-lasting for at least 36 months. MSCs and other regenerative cells contained in SVF produce many of reparative factors with immunomodulatory ability and after the injection they typically migrate and reside in soft synovial tissue of the target joint. Also, they may adhere to chondral cells in the lesions and start repair processes by production of new cartilage.[16]

To date, several reports regarding ADSCs therapy of degenerative OA were described. Many of them analyzed relatively small number of patients. The only large study using adipose tissue-derived stem cells is our previous clinical observation in the cohort of adult patients with grade 2–4 degenerative OA.[19] We described treatment of 1856 joints in 1128 patients and demonstrated the safety of SVF therapy and clinical improvement in a vast majority of patients without serious side effects.[19] Jo et al.[40] demonstrated that high-dose of ADSCs (1.0×10^8 versus 1.0×10^7 cells) injected intraarticularly in patients with knee OA reduced cartilage defects by regeneration of hyaline-like articular cartilage better than low-dose of the cells. In agreement with our previous study,[20] we used $14.4–40.1 \times 10^6$ SVF cells. Here we confirm that more than 15×10^6 SVF cells lead to long-lasting clinical effect.

Many patients in our study used large amounts of analgesics or non-steroidal anti-inflammatory drugs for a long period of time before SVF therapy. Pain and the amount of painkillers were significantly decreased after SVF therapy. In addition, similar results were obtained by evaluation of limping at walk, extent of joint

movement and joint stiffness (data not shown) and analyzed as complex Score evaluation of all 5 parameters (pain, number of analgesics/NSAIDs per week, limping at walk, joint stiffness, and extent of joint movement).

One of the limitations of our study is no randomization and placebo control mainly due to ethical aspects of autologous stem cell therapy. On the other hand, our large patient population can serve as the example that well designed case-control study represents safe, ethical, cost-effective and objective evaluation.

In conclusion, this is the first clinical observation clearly demonstrating benefits of SVF therapy in patients older than 80 years with grade 2–4 degenerative OA. Autologous SVF therapy of degenerative osteoarthritis is rapid, effective and safe method which improves significantly the quality of life in elderly patients with medium to advanced grade osteoarthritis.

Conflicts of interest

The authors declare no conflict of interest.

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