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ORIGINAL PAPER

The use of intra-articular injection of autologous micro-fragmented adipose tissue as pain treatment for ankle osteoarthritis: a prospective not randomized clinical study

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Abstract

Purpose The objective of this study was to evaluate the safety and the efficacy of intra-articular injections of autologous micro-fragmented adipose tissue in patients affected by early or moderate ankle osteoarthritis (AOA).

Methods A total of 31 symptomatic patients, aged 28–71 years, affected by AOA, were treated with 5 ml of autologous micro-fragmented adipose tissue. Clinical evaluations before the treatment and after six, 12, and 24 months were performed through American Orthopaedic Foot and Ankle Society (AOFAS) scale, the Foot and Ankle Disability Index (FADI), and Visual Analogue Scale (VAS) scores. Adverse events were recorded.

Results No severe complications were noted during the treatment and the follow-up period. A statistically significant improvement from basal evaluation to the six, 12-, and 24-month follow-up visit was observed, whereas a statistically significant worsening from the 12-month to the 24-month follow-up visit was showed.

Conclusion The autologous micro-fragmented adipose tissue for the treatment of pain in ankle osteoarthritis seems safe and able to provide positive clinical outcomes, potentially offering a new minimally invasive therapeutic option for patients who are not eligible for more invasive approaches. Further high-quality studies are needed to confirm these findings.

Keywords Ankle osteoarthritis · Autologous micro-fragmented adipose tissue · Prospective study · Adipose-derived mesenchymal stem cells

Introduction

Ankle osteoarthritis (AOA) is rarely idiopathic. Numerous clinical and epidemiological studies have pointed out that previous trauma represent the most common cause of osteoarthritis [1]. Malleolar fractures, ankle sprains in sports, congenital or acquired deformity, rheumatic diseases, cartilage, and osteochondral lesion are identified as main risk factors for AOA development. Therefore, patients affected by AOA are generally younger with high functional request [2–6]. AOA is heavily debilitating, and guidelines of treatment are not currently available. Non-operative treatments consist of anti-inflammatory drugs, physical therapy, and intra-articular injections (steroid, viscosupplementation, or platelet-rich plasma) [7]. However, the published data are limited and confined for early AOA [8]. Recently, a great interest about biological procedures in early degenerative disease has aroused. Intra-articular injection of adipose-derived mesenchymal stem cells (ADMSCs) represents an innovative approach to treat patients with knee OA. ADMSCs are traditionally obtained after enzymatic digestion and prolonged expansion in vitro [9]. However, their use is strictly regulated by complicated legislation. Therefore, in the last years, several strategies have been developed for ADMSCs use, resulting in the currently crucial concept of "minimal manipulation" [10]. The majority of these strategies are based on the principle that ADMSCs are contained in the micro-fragmented adipose tissue and do not need to be isolated to be activated. The micro-fragmented adipose

Level of Evidence: II, prospective cohort study

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tissue could be obtained by mechanical disaggregation of lipoaspirate and represents the ideal environment for cell preservation and activation [11–15]. Intra-articular injection of micro-fragmented adipose tissue represents an innovative approach to treat patients with knee OA. Indeed, the short-term clinical evaluation on knee osteoarthritis appears very promising [16–18]. On the other hand, to the best of our knowledge, only one report retrospectively analyzed the effect of arthroscopic debridement and autologous micronized adipose tissue injection in the treatment of advanced stage AOA [19]. The purpose of the present study was to prospectively assess the safety, clinical effectiveness, and feasibility of intra-articular injections of micro-fragmented adipose tissue in unilateral post-traumatic AOA in order to assess the improvement of symptoms and delay the necessity for invasive surgical procedures.

Materials and methods

This prospective open-label, single-centre, uncontrolled, pilot study was conducted with the highest respect for individual participants. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the revision of the Declaration of Helsinki, 2014. Before the beginning of any study-related activities, each study participant signed informed consent. The present study was approved by the Ethics Committee of Verona and Rovigo, Italy (protocol n. 61,386–19/09/2018).

Inclusion and exclusion criteria were summarized in Table 1. Standard weight-bearing anteroposterior and lateral views of the foot and mortise view of the ankle were performed to determine the grade of osteoarthritis by Kellgren-Lawrence (KL) classification (grade 1, doubtful narrowing of joint space and possible osteophytic lipping; grade 2, definite osteophytes and possible narrowing of joint space; grade 3, moderate multiple osteophytes, definite narrowing of joint space; and grade 4, large osteophytes, marked narrowing of joint space, severe sclerosis, and definite deformity of bone contour). Patients were evaluated before the treatment and prospectively after six, 12, and 24 months from the injection. American Orthopaedic Foot and Ankle Society (AOFAS) scale score, the Foot and Ankle Disability Index (FADI) score, and Visual Analogue Scale (VAS) scores were used for clinical evaluations. Adverse events were also recorded. Baseline characteristics (age, sex, weight, height, employment status as light workers or heavy labourers, side of involvement, and disease duration) were recorded before the first injection.

Harvesting the adipose tissue

The patient was placed supine. After local anaesthesia, a small incision was made to insert a 17G blunt cannula (connected to a luer-lock 60-cc syringe), and Klein sterile solution (containing saline, lignocaine, and epinephrine) injected into the subcutaneous fat. Approximately, 150–200 ml of this solution was injected in 50-ml aliquots into the lower abdominal area. Adipose tissue (approximately 50 ml) was then harvested manually via a 13G blunt cannula connected to the syringe. The area of fat harvest was tailored to the body habitus of each patient (normally lower abdomen or flank areas).

Processing the lipoaspirate and injecting the micro-fragmented adipose tissue

The lipoaspirate was processed using the Lipogems® system [20] following manufacturers' instruction. This is a disposable and single-use device constituted by a transparent plastic cylindrical container with stainless steel ball. The device is prefilled with saline. The lipoaspirate is introduced in a closed and aseptic manner. Through mechanical agitation of the container, the balls mechanically fragment the fat. The chamber is then flushed with saline to wash out impurities. The resulting product is then filtered through a 500-µm micron filter to obtain the micro-fragmented adipose tissue.

The skin was sterilely dressed, and the infiltration was performed through an anteromedial approach with a 22-gauge needle under ultrasound guidance (15–6 MHz

Table 1 Inclusion and exclusion criteria

Inclusion criteria	*Exclusion criteria*
Age > 18 years old	Systemic cardiovascular and coagulation disorders or anticoagulant therapy
Degenerative joint changes (KL 1–3)	Degenerative joint changes (KL 4)
Failure of previous conservative treatment (anti-inflammatory, physical therapy, intra-articular steroid, viscosupplementation, and platelet-rich plasma)	Intra-articular steroid or viscosupplementation injections performed within the last 3 months
History of chronic (≥ 4 months) pain or ankle swelling with limitation of daily activities	Rheumatic diseases and septic ankle arthritis
Completed follow-up	Cutaneous infection in abdomen and/or ankle

linear transducer). All patients received 5 cc of micro-fragmented adipose tissue and a series of instructions after every injection. In case of ankle pain during the treatment, they were recommended to use cold therapy and to rest for at least 24 hours. Otherwise, mild activities and a gradual resumption of normal sport or recreational activities were allowed as tolerated. An abdominal binder was then applied to the adipose tissue harvest site for two weeks.

The Student's *t* test was performed for AOFAS, FADI scores, and VAS to compare pre-operative and post-operative values. Data are expressed as a mean, standard deviation (SD), and 95% confidence interval (CI), and $p < 0.05$ was considered significant for one-tailed tests. The statistical software SPSS (version 17.0) was used for biometric analysis.

Results

From September 2018 to January 2019, 45 patients were assessed for eligibility. The study sample consisted of 31 patients. Patient's characteristics were summarized in Table 2.

The Student's *t* test was performed for AOFAS, FADI and EQ VAS scores to find statistically significant differences ($p < 0.05$ for one-tailed tests) comparing pretreatment with after six months, pretreatment with after 12 months, pretreatment with after 24 months, six months with 12 months results, six months with 24 months results, and 12 months with 24 months results.

The results showed that the micro-fragmented adipose tissue was statistically effective (Table 3 and Fig. 1). In fact, for AOFAS values, the results were significant at $p < 0.0005$ after six months ($t = 6.83$, $n = 31$), after 12 months ($t = 7.12$, $n = 31$), and after 24 months ($t = 2.35$, $n = 31$) (Table 3 and Fig. 1).

Moreover, the same statistically significance was found for FADI scores after six months ($t = 4.85$, $n = 31$), after 12 months ($t = 6.03$, $n = 31$), and after 24 months ($t = 3.39$ $n = 31$) using the same test.

Furthermore, for EQ VAS values, the related *t* test on the data, after six months ($t = 13.90$, $n = 31$), after 12 months ($t = 18.19$ $n = 31$), and after 24 months ($t = 9.09$, $n = 31$), showed significant results at $p < 0.005$ (Table 3).

A significant worsening between follow-up at 12 and 24 months was also assessed using the related *t* test. In fact, for AOFAS ($t = 4.86$, $n = 31$), FADI ($t = 3.45$, $n = 31$), and EQ VAS scores ($t = 4.58$, $n = 31$) obtained at 12 and 24 months, the results were significant at $p < 0.005$ (Table 3).

No severe complications related to the infiltrations were observed during the treatment and the follow-up period. Only minor side effects were detected in five patients (16%), such as transitory intra-articular burning sensation immediately after the injection or mild articular pain for a few days.

Discussion

The present study originally evaluated the safety and efficacy of autologous micro-fragmented adipose tissue in the management of symptoms of AOA. To the best of our knowledge, we found only one recent report that evaluates the efficacy of intra-articular injection of micro-fragmented adipose tissue in the treatment of the painful ankle. However, the authors considered only late stage of AOA and the micro-fragmented adipose tissue injections were included with an ankle arthroscopy and debridement. Therefore, any clinical results are unable to be attributable solely to the injection [19]. Although it could be argued that what is needed is an appropriate randomized clinical trial to assess the clinical effectiveness of micro-fragmented adipose tissue in AOA treatment, the presented pilot step is essential.

Table 2 Patient's characteristics

Characteristics	Ankle OA ($N = 31$)	Range
Age (years)	51.04 (15.49)	28–71
Sex (F/M)	12/19	
Weight (kg)	67.4 (9.6)	48–87
Height (cm)	168.8 (5.8)	158–188
Employment status (light worker/heavy labor)	18/13	
Etiology of OA (idiopathic/traumatic)	4/27	
Radiographic stage (Kellgren-Lawrence)		
Grade I	3	
Grade II	15	
Grade III	13	
Disease duration, years (SD)	6.4 (2.3)	4–9
Side of ankle OA (L/R)	14/17	

Data are mean (standard deviation, SD); F, female; M, male; L, left; R, right

Table 3 Global AOFAS, FADI, and EQ VAS scores at basal (PRE), 6-month (6 MO), 12-month (12 MO), and 24-month (24 MO) evaluations after treatment

Variable	Follow-up	Values		*t* value	*p*
AOFAS	PRE	56.4 (17.52)	PRE versus 6 MO	6.83	< 0.005
	6 MO	80.97 (9.01)	PRE versus 12 MO	7.12	< 0.005
	12 MO	84.17 (12.23)	PRE versus 24 MO	2.35	< 0.005
	24 MO	66.5 (15.71)	6 MO versus 12 MO	1.15	0.25
			12 MO versus 24 MO	4.86	< 0.005
FADI	PRE	59.47 (16.99)	PRE versus 6 MO	4.85	< 0.005
	6 MO	75.05 (8.78)	PRE versus 12 MO	6.03	< 0.005
	12 MO	82.0 (11.39)	PRE versus 24 MO	3.39	< 0.005
	24 MO	71.83 (10.88)	6 MO versus 12 MO	2.13	0.04
			12 MO versus 24MO	3.45	0.001
VAS	PRE	7.03 (0.95)	PRE versus 6 MO	13.90	< 0.005
	6 MO	3.61 (0.92)	PRE versus 12 MO	18.19	< 0.005
	12 MO	3.26 (0.63)	PRE versus 24 MO	9.09	< 0.005
	24 MO	4.35 (1.25)	6 MO versus 12 MO	1.96	0.06
			12 MO versus 24MO	4.58	< 0.005

Statistically significant differences ($p < 0.05$ for one-tailed tests) from basal evaluation to the follow-up visits were assessed using the Student's *t* test

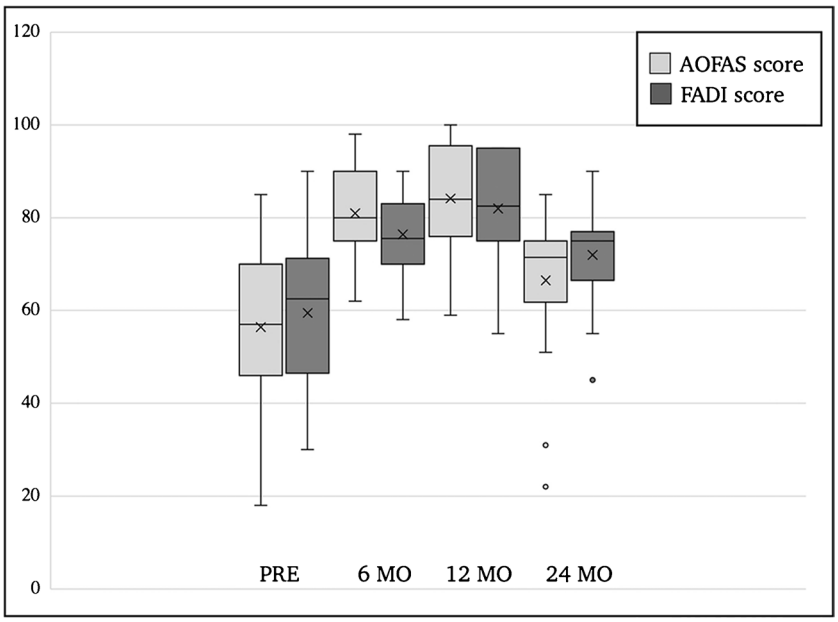

Fig. 1 Health status evaluated with AOFAS and FADI scores (0 to 100). Using the Student's *t* test and considering $p < 0.05$ significant for a one-tailed test, statistically significant improvements from basal evaluation to the six, 12- and 24-month follow-up visits were observed, whereas a significant worsening from the 12- to 24-month follow-up visits was noted. Black line, median; cross, mean; box limit, quartiles; extreme values, minimum–maximum; PRE, pretreatment; MO, months; AOFAS American Orthopaedic Foot and Ankle Society score; FADI, Foot and Ankle Disability Index score

The most important finding of the present research is that autologous micro-fragmented adipose tissue is a safe and effective treatment of pain and ankle function in AOA. Regarding our primary aim, we observed no severe complications related to the injections during the treatment and follow-up period, but only minor side effects were also common to other infiltration therapies [21].

Moreover, we found a positive effect on ankle function at short and medium follow-up, adding promising results to the available evidence about the use of micro-fragmented adipose tissue on clinical symptoms of osteoarthritis.

Indeed, both in vitro and in vivo studies reported that the micro-fragmented adipose tissue contains stromal and stem cells characterized by anti-inflammatory and regenerative properties through a plethora of bioactive elements as growth factors and cytokines [22].

It is relevant to highlight that all the patients included in the study had just been treated with other conservative therapies as physical therapy, medications, PRP, and HA infiltration without significant benefits. Therefore, autologous micro-fragmented adipose tissue may represent

another option to treat AOA in order to postpone invasive procedures especially in younger patients.

From our results, it is noted that the duration of treatment effectiveness lasts six months or up to one year with gradual worsening over a longer time similarly to viscosupplementation treatment [21]. Indeed, we began to observe a decrease of clinical improvement by 12 months, which may represent the start of a diminishing benefit from the treatment. It is unclear whether these patients' symptoms would continue to worsen to pre-injection levels or remain at this improved, but less than maximal, level. However, the improvement of symptoms even for just 12 months must be considered a success.

The present study has several limitations. One is the absence of a control group. However, we aimed to report a first pilot study. The results were not analyzed on the basis of age, severity of OA, pre-injection functional levels, or body mass index (BMI) because the numbers of patients studied were relatively small. The post-injection period was also not controlled for subject activity level or assistive devices usage. These variables might establish which patients would benefit most from this treatment and might help the physician to determine the best overall treatment plan for these patients.

In conclusion, the intra-articular injection of microfragmented adipose tissue represents a safe and effective treatment for AOA symptoms, offering a low-demanding and minimally invasive therapeutic option for patients who are not eligible for more invasive approaches as arthrodesis or arthroplasty. Further high-quality studies are needed to confirm these findings.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by L. Farinelli, S. Natali and V. Vacca. Statistical analysis was performed by V. Iacono. The first draft of the manuscript was written by L. Farinelli and D. Screpis. A. Gigante and C. Zorzi commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability Data are available if required.

Declarations

Ethical approval The present study was approved by the Ethics Committee of Verona and Rovigo, Italy (protocol n. 61386–19/09/2018).

Conflict of interest Each author certifies that he or she has no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted article.

References

1. Barg A, Pagenstert GI, Hügle T et al (2013) Ankle osteoarthritis: etiology, diagnostics, and classification. Foot Ankle Clin 18:411–426
2. Brown TD, Johnston RC, Saltzman CL, et al (2006) Posttraumatic osteoarthritis: a first estimate of incidence, prevalence, and burden of disease. In: Journal of Orthopaedic Trauma. J Orthop Trauma
3. Valderrabano V, Hintermann B, Horisberger M, Tak SF (2006) Ligamentous posttraumatic ankle osteoarthritis. Am J Sports Med 34:612–620. https://doi.org/10.1177/0363546505281813
4. Valderrabano V, Horisberger M, Russell I et al (2009) Etiology of ankle osteoarthritis. Clin Orthop Relat Res 467:1800–1806. https://doi.org/10.1007/s11999-008-0543-6
5. Daniels T, Thomas R (2008) Etiology and biomechanics of ankle arthritis. Foot Ankle Clin 13:341–352
6. Thomas RH, Daniels TR (2003) Ankle arthritis. J Bone Jt Surg Ser A 85:923–936
7. Witteveen AGH, Hofstad CJ, Kerkhoffs GMMJ (2015) Hyaluronic acid and other conservative treatment options for osteoarthritis of the ankle. Cochrane Database Syst. Rev. https://doi.org/10.1002/14651858.CD010643.pub2
8. Repetto I, Biti B, Cerruti P et al (2017) Conservative treatment of ankle osteoarthritis: can platelet-rich plasma effectively postpone surgery? J Foot Ankle Surg 56:362–365. https://doi.org/10.1053/j.jfas.2016.11.015
9. Orozco L, Munar A, Soler R et al (2013) Treatment of knee osteoarthritis with autologous mesenchymal stem cells: a pilot study. Transplantation 95:1535–1541. https://doi.org/10.1097/TP.0b013e318291a2da
10. Zellner J, Johnstone B, Barry F, Madry H (2019) Mesenchymal stem cell based regenerative treatment of the knee: from basic science to clinics. Stem Cells Int 2019:1–1. https://doi.org/10.1155/2019/7608718
11. Senesi L, De Francesco F, Farinelli L, et al (2019) Mechanical and enzymatic procedures to isolate the stromal vascular fraction from adipose tissue: preliminary results.Front Cell Dev Biol 7.https://doi.org/10.3389/fcell.2019.00088
12. De Francesco F, Ricci G, D'Andrea F et al (2015) Human adipose stem cells: from bench to bedside. Tissue Eng Part B Rev 21:572–584
13. De Francesco F, Matta C, Riccio M et al (2021) Reevolution of tissue regeneration: from recent advances in adipose stem cells to novel therapeutic approaches. Stem Cells Int 2021:1–3. https://doi.org/10.1155/2021/2179429
14. Prè ED, Busato A, Mannucci S, et al (2020) In vitro characterization of adipose stem cells non-enzymatically extracted from the thigh and abdomen.Int J Mol Sci 21.https://doi.org/10.3390/ijms21093081
15. Busato A, De Francesco F, Biswas R, et al (2020) Simple and rapid non-enzymatic procedure allows the isolation of structurally preserved connective tissue micro-fragments enriched with SVF. Cells 10.https://doi.org/10.3390/cells10010036
16. Russo A, Condello V, Madonna V et al (2017) Autologous and micro-fragmented adipose tissue for the treatment of diffuse degenerative knee osteoarthritis. J Exp Orthop 4:33. https://doi.org/10.1186/s40634-017-0108-2
17. Russo A, Screpis D, Di Donato SL, et al (2018) Autologous microfragmented adipose tissue for the treatment of diffuse degenerative knee osteoarthritis: an update at 3 year follow-up.J Exp Orthop 5.https://doi.org/10.1186/s40634-018-0169-x
18. Tsubosaka M, Matsumoto T, Sobajima S et al (2020) The influence of adipose-derived stromal vascular fraction cells on the treatment of knee osteoarthritis. BMC Musculoskelet Disord 21:207. https://doi.org/10.1186/s12891-020-03231-3

19. Shimozono Y, Dankert JF, Kennedy JG (2020) Arthroscopic debridement and autologous micronized adipose tissue injection in the treatment of advanced-stage posttraumatic osteoarthritis of the ankle. Cartilage. https://doi.org/10.1177/1947603520946364
20. Bianchi F, Maioli M, Leonardi E et al (2013) A new nonenzymatic method and device to obtain a fat tissue derivative highly enriched in pericyte-like elements by mild mechanical forces from human lipoaspirates. Cell Transplant 22:2063–2077. https://doi.org/10.3727/096368912X657855
21. Sun SF, Chou YJ, Hsu CW et al (2006) Efficacy of intra-articular hyaluronic acid in patients with osteoarthritis of the ankle: a prospective study. Osteoarthr Cartil 14:867–874. https://doi.org/10.1016/j.joca.2006.03.003
22. Chamberlain G, Fox J, Ashton B, Middleton J (2007) Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells 25:2739–2749. https://doi.org/10.1634/stemcells.2007-0197