Comparative Clinical Outcomes After Intra-articular Injection With Adipose-Derived Cultured Stem Cells or Noncultured Stromal Vascular Fraction for the Treatment of Knee Osteoarthritis

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Background: Intra-articular injection of adipose-derived stem cells (ASCs) has shown promise for improving symptoms and cartilage quality in the treatment of osteoarthritis (OA). However, while most preclinical studies have been performed with plastic-adherent ASCs, most clinical trials are being conducted with the stromal vascular fraction (SVF), prepared from adipose tissue without prior culture.

Purpose: To directly compare clinical outcomes of intra-articular injection with ASCs or SVF in patients with knee OA.

Study Design: Cohort study; Level of evidence, 3.

Methods: The authors retrospectively compared 6-month outcomes in 42 patients (59 knees) receiving intra-articular injection with 12.75 million ASCs and 38 patients (69 knees) receiving a 5-mL preparation of SVF. All patients had Kellgren-Lawrence grade 2, 3, or 4 knee OA and had failed standard medical therapy. The visual analog scale (VAS) pain score and Knee injury and Osteoarthritis Outcome Score (KOOS) at baseline and 1, 3, and 6 months after injection were considered as outcomes. Outcome Measures in Rheumatology–Osteoarthritis Research Society International (OMERACT-OARSI) criteria were also used to assess positive response. A repeated measures analysis of variance was used for comparison between the treatment groups.

Results: No major complications occurred in either group. The SVF group had a higher frequency of knee effusion (SVF 8%, ASC 2%) and minor complications related to the fat harvest site (SVF 34%, ASC 5%). Both groups reported improvements in pain VAS and KOOS domains. Specifically, in the ASC group, symptoms improved earlier (by 3 months; $P < .05$) and pain VAS decreased to a greater degree (55%; $P < .05$) compared with the SVF group (44%). The proportion of OMERACT-OARSI responders in the ASC group was slightly higher (ASCs, 61%; SVF, 55%; $P = .25$).

Conclusion: It was observed that both ASCs and SVF resulted in clinical improvement in patients with knee OA, but that ASCs outperform SVF in the early reduction of symptoms and pain with less comorbidity.

Keywords: adipose-derived stem cells; stromal vascular fraction; knee osteoarthritis; intra-articular injection

Japan has the world's highest proportion of citizens over 65 years (27%),[21] making osteoarthritis (OA) one of the most important challenges in the nation's efforts to realize a healthy and able-bodied society. Currently, there are no existing disease-modifying therapies that address structural abnormalities of the knee or other joints affected by OA. The purpose of all existing therapies, including surgical treatment, is symptom improvement, mainly to mitigate pain. However, we and others have reported that the intra-articular injection of adipose-derived stem cells (ASCs) may improve cartilage quality and delay OA progression.[2,7,10,12,14-16,20,22] First described in 2001, ASCs are attractive because, relative to bone marrow–derived cells, they are easier to obtain and may include a higher proportion of mesenchymal stem cells.[3,12,15,24] Currently, in the US, there are 7 active clinical trials examining the safety and/or efficacy of ASCs for treating OA.[4]

A major limitation of the current clinical trials is that they exclusively use the stromal vascular fraction (SVF), rather than cultured, plastic-adherent ASCs, due to restrictions on the use of cultured cells in humans. Like ASCs, the SVF is harvested from adipose tissue but is administered directly after tissue digestion and lavage of

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TABLE 1
Inclusion and Exclusion Criteria[a]

Inclusion Criteria	Exclusion Criteria
Provide written informed consent	Unable to provide consent
Knee OA confirmed by clinical evaluation and MRI	Patient unable to undergo MRI
Kellgren-Lawrence grade 2, 3, or 4	Any form of inflammatory arthritis
ASA class 1 or 2	ASA class 3 or higher
No other comorbidities	Comorbidities, including hemotherapy or within 5 years of cancer treatment; hepatitis B or C virus; human immunodeficiency virus; syphilis
	Hyaluronic acid or steroid injection treatment within 3 months

[a]ASA, American Society of Anesthesia; MRI, magnetic resonance imaging; OA, osteoarthritis.

the liberated cells, without cell culture. While convenient, this means that the step in which the heterogeneous SVF cell population (including hematopoietic, vascular, and stromal cells) is plated to select for plastic-adherent mesenchymal stem cells is bypassed.[3,11] This is particularly problematic as characterization of the chondrogenic potential of ASCs, demonstration of efficacy in experimental models, and human dose escalation studies (completed outside the US) have all been performed with cultured, plastic-adherent ASCs.[7,15,16,20] Moreover, plastic adherence is one of the defining properties of mesenchymal stem cells, whether they are derived from adipose or other tissues.[6] The use of SVF for OA is not unprecedented. We and others have shown that SVF can improve symptoms and cartilage quality.[10,15,22] However, the relative therapeutic value of intra-articular injection of SVF compared with ASCs for OA is unknown.

The purpose of our study was to directly compare the clinical outcomes of intra-articular injection with ASCs or SVF in patients with knee OA. While the clinical administration of cultured cells is not possible in the US, in Japan the Cell Source Center for Regenerative Medicine is able to prepare cultured cells for clinical use under the Regenerative Medicine Promoting Act on the Safety of Regenerative Medicine. Enacted in May 2013, this legislation was the world's first national framework to promote regenerative medicine in clinical studies and private practice. On the basis of this vanguard national initiative, we were permitted to perform 2 retrospective studies comparing the 6-month outcomes of patients receiving ASCs or SVF for symptomatic knee OA.

METHODS

In accordance with the Regenerative Medicine Promoting Act on the Safety of Regenerative Medicine, the study "Joint Treatment by Injecting Adipose Tissue-Derived Cultured Stem Cells" was submitted by our clinic and accepted at the Bureau of Health and Welfare of the Ministry of Health, Labour and Welfare after review by the Committee of Specified Accreditation of Regenerative Medicine. This study was also approved by our hospital's ethical review committee (institutional review board [IRB]). Due to the vanguard nature of this clinical research, a randomized controlled trial was not approved, and the IRB requested that 6-month follow-up results be evaluated for safety and effectiveness. Instead, we were asked to complete a retrospective cohort study. The SVF protocol was approved by our IRB in 2016 and the ASC in 2017, with the ASC protocol also approving comparison of these 2 cohorts.

An a priori power calculation revealed that we would need 36 knees per group to achieve 80% power to detect an effect size of 0.25 in the pain visual analog scale (VAS), which we considered the primary outcome, while 55 knees per group were deemed necessary to achieve 80% power for a minimal clinically important change in the Knee injury and Osteoarthritis Outcome Score (KOOS) subscales, which we considered the secondary outcomes.

Therefore, we reviewed the outcomes of 80 patients (128 knees), who received intra-articular injection using ASCs or SVF at our clinic and completed 6 months of posttreatment follow-up (Table 1). Per protocol, patients seen between November 2016 and April 2017 were treated with SVF, while patients seen between May 2017 and October 2017 received ASCs. Patients were included if they had a clinical diagnosis of knee OA, which was a Kellgren-Lawrence (KL)[9] grade 2, 3, or 4, that was confirmed by radiograph.

All patients had failed nonoperative medical treatment. Specifically, patients had been treated with oral nonsteroid anti-inflammatory drugs and intra-articular hyaluronic

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TABLE 2
Characteristics of Patients Undergoing Intra-articular Injection of ASCs or SVF Cells for Knee Osteoarthritis[a]

Characteristic	ASC (n = 42, 59 knees)	SVF (n = 38, 69 knees)
Mean age, y	70 ± 9.1	73 ± 9.1
Male/female	9/33	7/31
Mean height, cm	156.8 ± 7.7	155.7 ± 8.4
Mean weight, kg	61.4 ± 10.9	60.7 ± 11.4
Mean BMI, kg/m^2	24.8 ± 2.0	24.8 ± 1.9
Kellgren-Lawrence grade		
2	4	4
3	25	26
4	13	8
Mean duration since onset, y	6.9 ± 5.0	7.4 ± 6.4
Previous knee arthroscopy	6	6
Previous hyaluronic acid injection (+)	39	35
Previous steroid injection (+)	3	2
MRI concomitant ligament injuries (+)	18	16
Meniscal lesions (+)	38	37
Bone marrow edema lesions (+)	33	30
Cells injected, millions	12.75 ± 2.9	Unknown

[a]Values are given as number or mean ± SD. $P > .05$, Mann-Whitney test. ASC, adipose-derived stem cell; BMI, body mass index; MRI, magnetic resonance imaging; SVF, stromal vascular fraction.

acid and/or steroid injection, but due to poor response, most of the cases had been recommended for total knee arthroplasty (TKA) by the previous doctors. Due to the varied nature of referral, we were unable to reliably measure the time since failed treatments. The patients came to our clinic at this stage understanding that our clinic would offer experimental medical treatment. Concomitant pathologies, such as incidence of ligament injuries, meniscal lesions, and bone marrow edema, were evaluated by magnetic resonance imaging. All patient characteristics and clinical findings were balanced between the groups (Table 2).

Forty-two patients (59 knees) underwent ASC injection as described in the Appendix (available in the online version of this article). The other cohort of 38 patients (69 knees) received a single intra-articular injection of an SVF preparation. The method for harvesting the SVF was similar to that for the ASC, except that a greater quantity of liposuction was required (more than 100 mL for a single knee or more than 200 mL for both knees), and the cells were not cultured, but isolated and injected on the same day.[22] The number of cells injected was unknown for the SVF group as the entire 5 mL of SVF produced was subsequently injected.[11,22] All injections in both cohorts were performed by 2 senior orthopaedic surgeons (N.Y. and M.H.) with decades of experience performing unguided knee injections.

From the day of injection to 3 days after, all patients received oral analgesics (60 mg loxoprofen sodium hydrate as needed) and antibiotics (3-day course of cefcapene pivoxil hydrochloride hydrate). After the injection, patients rested in bed for 10 minutes before starting rehabilitation on the same day following instruction by a physical therapist in the clinic before discharge. Both groups underwent the same rehabilitation protocol: the only instruction was knee excursion training, which was to be performed at least 100 times a day.

The primary outcome was clinical outcomes as measured by patient-reported outcome measures (PROMs). The KOOS[18] and VAS score for pain were collected at baseline, before the injection, and 1, 3, and 6 months after injection. If a patient had symptoms in both knees, he or she completed the KOOS and VAS based on a perception of the knee with worse pain. We also used the Outcome Measures in Rheumatology–Osteoarthritis Research Society International (OMERACT-OARSI) set of responder criteria[17] to determine the effect of the injection. Total range of motion was collected at baseline and 6 months. Adverse events were recorded for safety evaluation.

For continuous variables, we report the mean and SD, while for discrete variables we report frequency counts and percentages. Hypothesis testing was conducted using the Mann-Whitney test, repeated measures analysis of variance, and Cochran-Mantel-Haenszel chi-square test with a critical P value of .05 denoting statistical significance. All analyses were performed using Statcel 4 (OMS Ltd).

RESULTS

Patients in the ASC and SVF groups were similar with no significant differences in characteristics or clinical presentation ($P > .05$) (Table 2).

The severity of OA was also similar between the groups: the KL grade for the majority of patients in both the ASC (60%) and SVF (68%) groups was 3 (midstage), and both groups also included 4 patients each in KL grade 2, with the remainder in KL grade 4 (Table 2). The degree of comorbidity, as assessed by the American Society of Anesthesiologists score, was also similar between the groups.[1]

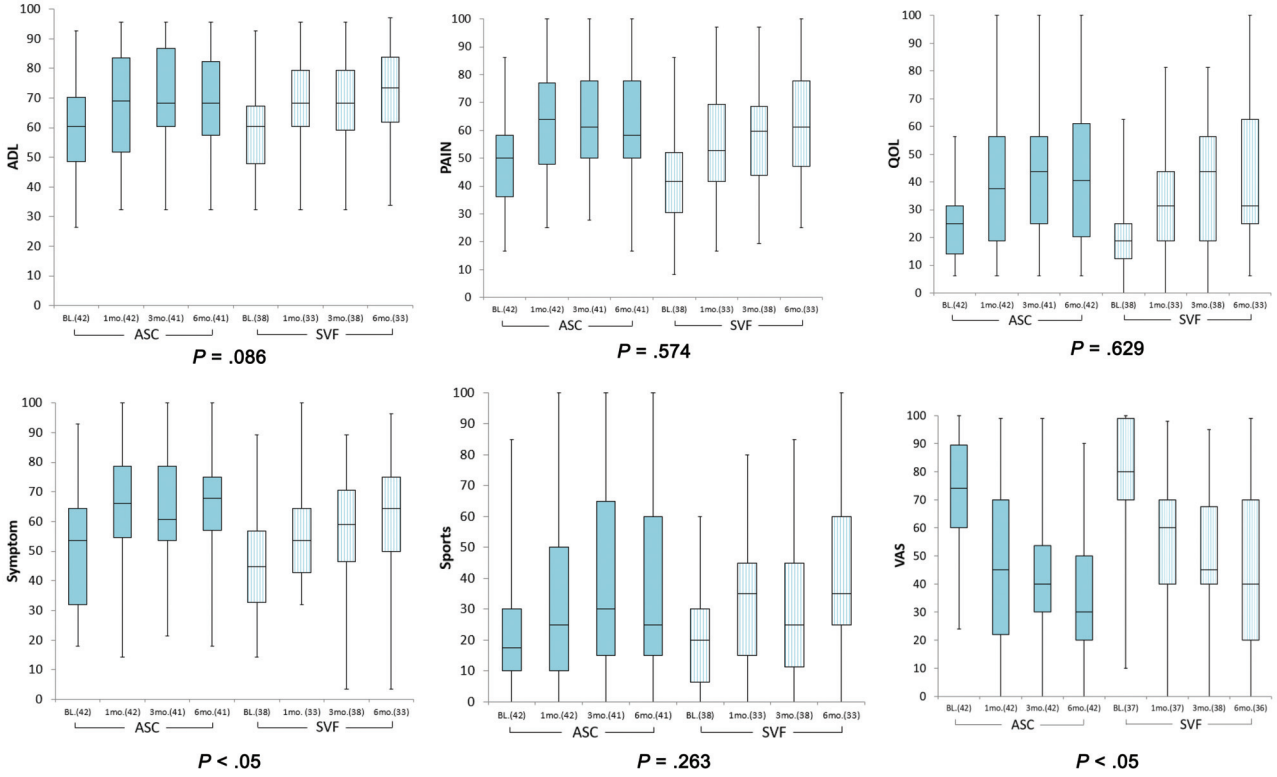

Figure 1. Knee injury and Osteoarthritis Outcome Score (KOOS) domains and pain visual analog scale (VAS) scores at baseline (BL) and 1, 3, and 6 months after intra-articular injection with adipose-derived stem cells (ASCs; n = 42) or stromal vascular fraction (SVF; n = 38). Patients treated with ASCs reported earlier improvement of KOOS symptoms and a greater decrease in VAS (pain) compared with patients receiving SVF. Significance (*P* values) was calculated by repeated measures analysis of variance. ADL, activities of daily living; QOL, quality of life.

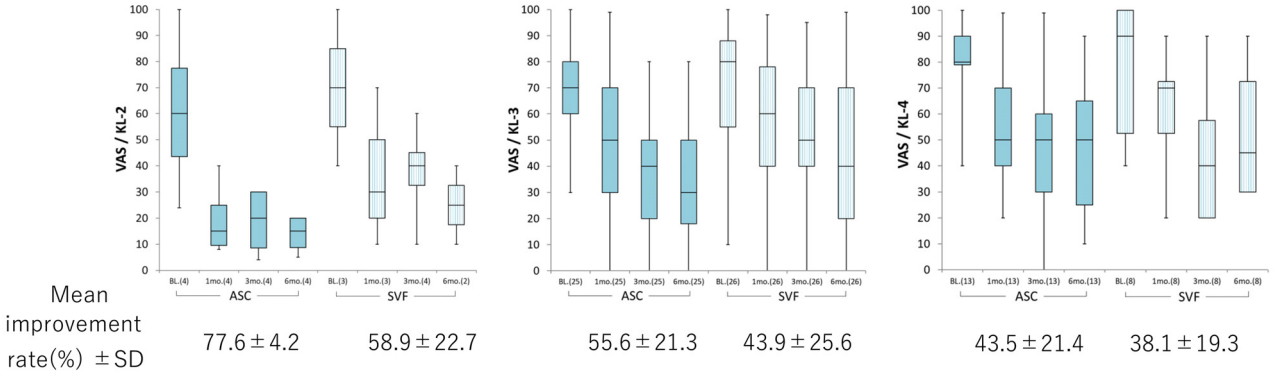

Figure 2. Pain visual analog scale (VAS) at baseline (BL) and 1, 3, and 6 months after intra-articular injection of adipose-derived stem cells (ASC) or stromal vascular fraction (SVF) cells for knee osteoarthritis, analyzed by Kellgren-Lawrence (KL) grade.

Scores for all domains of the KOOS were similar in the ASC and SVF groups at baseline and before injection (Figure 1).

Change in KOOS symptoms occurred earlier in the ASC group than the SVF group, with significant improvement detected at 3 months after injection (Figure 1). Other KOOS domains were similar in the 2 groups. Pain VAS declined earlier and to a greater extent in the ASC group (54.6% ± 21.7%) than the SVF group (44.0% ± 26.1%; $P < .05$) (Figure 1). Analysis of VAS by KL grade showed increasing baseline VAS score with worsening radiographic OA, as expected (Figure 2).

The extent of VAS improvement after injection was greatest in patients with mildest OA (KL grade 2) and

TABLE 3
OMERACT-OARSI Responder Rate by KL Grade in Patients Receiving Intra-articular Injection of ASCs or SVF Cells[a]

KL Grade	ASC (n = 42)	SVF (n = 38)
2	100% (4/4)	50% (2/4)
3	64% (16/25)	58% (15/26)
4	46% (6/13)	50% (4/8)
Total	62% (26/42)	55% (21/38)

[a]$P > .05$, Cochran-Mantel-Haenszel chi-square test. ASC, adipose-derived stem cell; KL, Kellgren-Lawrence; OMERACT-OARSI, Outcome Measures in Rheumatology–Osteoarthritis Research Society International; SVF, stromal vascular fraction.

decreased with worsening radiographic OA, regardless of treatment group (Figure 2). Regardless of KL grade, patients in the ASC group had a greater improvement in VAS than patients in the SVF group (Figure 2).

The proportion of patients who responded positively based on OMERACT-OARSI responder criteria was higher in the ASC group (61%) than the SVF group (55%) (Table 3), although this was not statistically significant ($P = .25$).

In the ASC group, the proportion of responders was greatest in patients with KL grade 2 (100%), and the proportion decreased as the KL grade (severity of OA) increased. In the SVF group, differences in the proportion of responders did not appear to differ based on KL grade. Baseline and 6-month total range of motion did not differ between the groups, with the difference between the groups at 6 months being just 0.9° (Appendix Table A1, available online).

No serious adverse events occurred in either group during the 6-month follow-up period. Mild knee effusion/swelling was observed in 1 case in the ASC group and 3 cases in the SVF group. One of the 3 cases in the SVF group was treated by 1-time knee aspiration, while all other cases resolved without intervention (Table 4).

In the SVF group there were 6 cases of abdominal pain and 5 cases of internal bleeding at the incision site 1 month after cell harvest, but all were resolved without intervention. Subcutaneous induration at the abdominal area fat harvest site was observed in 2 cases in the ASC group and 12 cases in the SVF group; however, they had all resolved without intervention by the 6-month final follow-up (Table 4).

TABLE 4
Adverse Events Occurring Within 6 Months After Intra-articular Injection With ASCs or SVF for Knee Osteoarthritis[a]

	Treatment	
	ASC (n = 42)	SVF (n = 38)
Serious adverse events[b]	0	0
Knee		
Infection	0	0
Effusion/swelling[c]	1 (2%)	3 (8%)
Abdomen (fat harvest site)[d]		
Infection	0	0
Pain	0	6 (16%)
Bleeding	0	5 (13%)
Induration	2 (5%)	12 (32%)
Total	2 (5%)	13 (34%)

[a]$P < .05$, Cochran-Mantel-Haenszel chi-square test. ASC, adipose-derived stem cell; SVF, stromal vascular fraction.
[b]Serious adverse events included death, hospitalization, disability, and cancer.
[c]All cases of knee effusion/swelling resolved without intervention within 6 months except 1 case in the SVF group that required aspiration.
[d]All adverse events at the abdominal fat harvest site resolved without intervention within 6 months.

DISCUSSION

We compared clinical outcomes 6 months after intra-articular injection with ASCs or SVF in patients with knee OA who had failed medical treatment and were indicated for TKA. Differences between treatment groups were small, but those receiving ASCs had a more rapid and greater improvement in pain and symptoms. A larger proportion of patients in the ASC group reported a positive response based on the OMERACT-OARSI set of responder criteria than patients in the SVF group. The proportion of responders also correlated with KL grade in the ASC group, but not in the SVF group. No serious complications occurred, but patients receiving SVF had a greater frequency of nonserious adverse events, including mild knee effusion and minor wound complications around the abdominal fat harvest site. Our study suggests that ASCs are as safe as and potentially more effective than SVF for intra-articular injection in patients with knee OA.

Our findings are consistent with those of previous studies that have reported on the safety of intra-articular injection of adipose-derived cells for knee OA.[7,10,12,14-16,22,23] The more frequent occurrence of knee swelling in the SVF group may be attributable to the presence of white blood cells in the heterogeneous SVF cell preparation.[3,11] The greater frequency of incisional pain, harvest site bleeding, and induration of the abdominal area in the SVF group was not surprising, given that as much as 10 times more fat was harvested for SVF (more than 200 mL for both knees) compared with ASC (20 mL). Neither group had any major complications and the ASC group had slightly lower minor adverse events, underscoring the safety of cultured adipose-derived cells, consistent with previous reports.[7,12,14-16] A recent paper reporting the transformation of human adipose–derived stem cells into cancer cells after long-term culture[19] was subsequently retracted upon discovery that the apparent transformation occurred due to cell contamination.[5] The theoretical risk of cancer based on treatment with ASC remains unknown but is likely minimal. With a national initiative like Japan's Regenerative Medicine Promoting Act on the Safety of Regenerative Medicine, it is apparent that protocols can be developed for safely administering cultured cells to human patients.

A limited number of previous studies have examined the effect of intra-articular ASC or SVF injection without additional carriers such as hyaluronic acid or platelet-rich plasma.[7,10,16,22] Our findings are in agreement with these previous reports, confirming the potential efficacy of the cells themselves, apart from any carrier. Two recent studies of SVF intra-articular injection reported improvements in pain VAS scores in a similar range observed in our patients.[10,22] One of these studies also reported an improvement in all KOOS domains 3 months to 2 years after SVF injection,[10] and this is consistent with our findings. We previously reported improved patient-reported outcomes using different measures (Western Ontario and McMaster Universities Arthritis Index [WOMAC], Japanese Knee Osteoarthritis Measure, and VAS).[22] Two recent studies of ASC intra-articular injection also reported findings similar to those of our study. Jo et al[7] reported a change in VAS scores from 79 $\pm$ 2 mm at baseline to 44 $\pm$ 6 mm at 6 months after ASC injection. A similar change in VAS (-41 ± -13) was reported by Pers et al.[16] In these dose escalation studies, one observed a significant change in pain only in the high-dose group receiving 10^8 cells (Jo et al), while the other observed it only in the low-dose group receiving 2×10^6 cells (Pers et al). Our study showed a similar extent of change in VAS after intra-articular injection with a dose between that of these 2 studies (1.25×10^7 cells). Dose-related differences may have arisen due to differences in cell preparation methods or in the quality of individual donors' cells. However, the effects on pain and symptoms suggest that optimization of ASC dosing may still be needed. In the study of Pers et al, the proportion of OMERACT-OARSI responders receiving a similar number of cells (1 or 5×10^7 cells) to patients in our study was 60% (vs 61% in our study), but it was higher (80%) in the group receiving the lowest dose of ASCs (10^6 cells). Patients receiving the lower doses in the study of Pers et al also reported improved KOOS scores, in agreement with our study. However, using measures that were different from our study (WOMAC and Knee Society Score), Jo et al also reported improved PROM scores.

The most important contribution of our study to the development of ASC-based therapy for OA is the direct comparison of ASCs and SVF. Direct comparison, which has not previously been reported, is important as most of the experimental models demonstrating efficacy have been performed with plastic-adherent ASCs, while most clinical studies in humans are being performed with SVF cells.[15] In our study, direct comparison shows that while both cell preparations improved pain and symptoms, ASCs outperform SVF. The scientific basis accounting for such a difference is not clear; however, it may be attributable to the lower proportion of hematopoietic cells and higher frequency of colony-forming unit fibroblasts (CFU-Fs) in ASCs compared with SVF.[3]

In an experimental model of OA, labeled ASCs were observed in the subintimal layer of the synovium after injection, suggesting that ASCs may protect joints by inhibiting macrophage activation.[20] In the experimental model, positive effects were only seen when ASCs were injected early in the progression of OA, which is consistent with our findings in which the proportion of responders was higher in the KL grade 2 and 3 patients treated with ASCs than in the KL grade 4 patients. In the ASC group, the extent of pain relief was also related to KL grading. For patients in the SVF group, neither was the case. While this may reflect the small number of patients with early OA in our study, it may also suggest that the mechanisms of action of SVF and ASC differ.

There are several limitations in the present study. Most importantly, we were unable to conduct this study in a blinded or randomized fashion. The 2013 Japanese Regenerative Medicine Promoting Act on the Safety of Regenerative Medicine allowed this study to be conducted, but we were not approved for a randomized design. We hope that these preliminary data will allow us to reapply with a well-designed randomized controlled trial now that we have demonstrated the safety and effectiveness of the therapies. Despite this limitation, the patient characteristics in each of our treatment groups were reasonably well balanced, so any differences between the groups may be attributable to the different treatments received. Blinding may not be possible when comparing ASC and SVF due to differences in surgical invasiveness of tissue harvest and the time required to prepare each cell type. Randomization, however, is reasonable, especially in the wake of our study, where even though the ASC treatment produced slightly better results, both groups did experience improvement in pain and symptoms.

Another limitation was the relatively short follow-up duration of only 6 months. A longer follow-up should provide more information regarding the safety and effectiveness of these 2 adipose tissue-derived cell therapies. On the other hand, considering the fact that ASC therapy is rare in the US and Europe, we felt compelled to report our early findings because there was already a significant difference in patients' symptoms, in addition to clear differences in the incidence of complications related to tissue harvest. Future studies should attempt to establish the longevity of ASC (or SVF) treatment, with follow-up for more than 6 months using conversion to TKA as a measure of therapeutic failure. Mitigation of pain can already be achieved through currently available medications or surgery. Reversing or preventing OA progression to delay or obviate TKA should be the goal of stem cell therapy. Only long-term studies will be able to establish the ultimate effectiveness of stem cell therapy for OA.

Finally, the present study did not morphologically evaluate change in the articular surface. Previous preclinical and clinical studies have reported improved cartilage quality after intra-articular ASC or SVF injection.[7,10,15,16] Although the mechanisms of this effect are unknown, previous studies using stem cells to repair discrete cartilage defects suggest that the presence of chondrogenic CFU-Fs may contribute.[8,13,15] Given that ASCs include a greater proportion of CFU-Fs than SVF,[3] and that ASCs improved defect repair somewhat better than SVF in an experimental model,[8] future radiographic or arthroscopic studies in our patients would be expected to show greater improvement in cartilage quality in the ASC group than the SVF group. We plan to evaluate our patients via radiograph at 2 and 5 years and will also follow them long term for progression to TKA.

CONCLUSION

Ours is the only existing study that directly compares the intra-articular injection of ASCs or SVF for knee OA, and we observed small but significant advantages of using ASCs. This is not surprising, given that most preclinical studies were conducted using ASCs,[15] and that plastic adherence is widely recognized as a defining characteristic of stem cells.[3,6] Our findings are in agreement with a previous cartilage defect repair study in sheep, where ASCs were slightly superior to SVF.[8] Our study showed improvements in pain and symptoms in more than half of patients receiving SVF, supporting the continued study of these cells in current clinical trials. Based on our findings, randomization between ASC and SVF would be reasonable. Our findings also strongly support the development of programs like Japan's act to ensure the safety of regenerative medicine so that cultured stem cells, including ASCs, can continue to be developed for the treatment of OA and other diseases.

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