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Concise report

Autologous adipose-derived stromal vascular fraction in patients with systemic sclerosis: 12-month follow-up

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Abstract

Objective. Impaired hand function greatly contributes to disability and reduced quality of life in SSc patients. Autologous adipose-derived stromal vascular fraction (ADSVF) is recognized as an easily accessible source of regenerative cells. We reported positive 6-month safety and efficacy results from an open-label clinical trial assessing s.c. injection of autologous ADSVF into the fingers in SSc patients. The objective of this report is to describe the effects at 12 months.

Methods. Twelve females, mean age 54.5 years (S.D. 10.3), were assessed 1 year after ADSVF injection. Patients were eligible if they had a Cochin Hand Function Scale score >20/90. ADSVF was obtained from lipoaspirate using an automated processing system and subsequently injected into the s.c. tissue of each finger in contact with neurovascular pedicles in a one-time procedure. Endpoints were changes in hand disability and skin fibrosis, vascular manifestations, pain and quality of life at the 12 month follow-up. During the visit, patients estimated the benefit of the procedure with a specific self-completed questionnaire.

Results. A significant decrease from baseline of 51.3% ($P < 0.001$) for Cochin Hand Function Scale score, 63.2% ($P < 0.001$) for RP severity and 46.8% ($P = 0.001$) for quality of life (Scleroderma Health Assessment Questionnaire) was observed. A significant improvement of finger oedema, skin sclerosis, motion and strength of the hands and of the vascular suppression score was also noted. The reduction in hand pain approached statistical significance ($P = 0.052$). The questionnaire revealed a benefit in daily activities, housework and social activities.

Conclusion. ADSVF injection is a promising therapy and appears to have benefits that extend for at least 1 year.

Key words: systemic sclerosis, stromal vascular fraction, hand, adipose tissue, cell therapy.

Trial registration: ClinicalTrials.gov (NCT01813279).

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Rheumatology key messages

- Hand involvement due to scleroderma leads to disability and impaired quality of life.
- Adipose-derived stromal vascular fraction (ADSVF) injection in the fingers of scleroderma patients can be performed safely.
- ADSVF injection in the fingers of scleroderma patients appeared to be associated with prolonged efficacy.

Introduction

Hand disability represents a large burden in work and daily activities for SSc patients [1, 2]. Vasodilators, lifestyle changes and physiotherapy are recommended, but remain of limited efficacy in improving patients' complaints. No anti-fibrotic treatment has proven effective [3]. Unlike other autoimmune diseases, immunosuppressive drugs have limited clinical interest. Thus functional improvement of hand motion represents a real challenge for physicians and a priority for patients. Identification and characterization of the adipose-derived stromal vascular fraction (ADSVF), which includes mesenchymal stem cell–like cells, endothelial progenitor cells and hematopoietic cells, have resulted in the recognition of adipose tissue as a source of cells with multipotency, angiogenic, reparative and immunomodulatory properties. The ADSVF may have potential efficacy against ischaemia and skin fibrosis, two major manifestations of SSc. We reported the safety and feasibility of the s.c. injection of autologous ADSVF in patients with SSc enrolled in the Scleradec trial (NCT01813279) [4]. In this trial we observed a significant improvement in quality of life, hand disability, pain, RP severity, digital ulcers (DUs), finger circumference and vascular suppression score (VSS) at the 6 month follow-up. Herein we report data from a 12-month follow-up in order to determine the long-term effect of this cell-based therapy in the same cohort.

Methods

Study population

Twelve females with a mean age of 54.5 years (S.D. 10.3, range 34.0–68.0) were assessed 1 year after ADSVF injection. The population mainly included patients with limited cutaneous SSc and without severe organ damage. The clinical and biological characteristics of the SSc patients and their ongoing treatments have been described previously [4]. All enrolled patients had a score $\geqslant 9$ according to the 2013 EULAR/ACR criteria for SSc [5] and had previously received optimal treatment for digital vasculopathy, according to EULAR recommendations [6]. Patients were eligible if they had a Cochin Hand Function Scale (CHFS) score $>20/90$, a questionnaire commonly used to assess hand disability in SSc [2]. Exclusion criteria were new vasodilators or immunosuppressive therapy for SSc in the 3 months prior to enrolment or during the follow-up; surgical contraindication; signs of digital infection; positive status for HIV, HBV or HCV, HTLV-1 or -2 or syphilis; pregnancy or BMI $<17\,kg/m^2$. Written informed consent was obtained according to the Declaration of Helsinki and the study has been approved by the local ethics committee review board of Marseilles.

Procedures

Adipose tissue collection and ADSVF injection were conducted under conscious sedation; harvesting areas were anaesthetized. Harvesting was performed with a 10 ml syringe in a closed circuit using a 3 mm Coleman cannula. Fat was harvested from various areas depending on the patient's morphology, i.e. lateral aspect of the hips, knees, flanks, thighs and abdomen. Once harvesting was complete, the bag was immediately transported to the registered cell therapy unit. Autologous ADSVF was obtained within 2 h after lipoaspiration using automated processing with a Celution 800/CRS system (Cytori Therapeutics, San Diego, CA, USA). ADSVF cells were injected using a 25-gauge (0.5×40 mm) reinforced cannula placed into the s.c. tissue in contact with the neurovascular pedicles: 0.5 ml of ADSVF was injected into each lateral side of each digit, using a retrotracing technique, from distal to proximal. Entry points were positioned at the MCP joint for the thumb and the PIP joint where the palmar and dorsal skin joins for the long fingers.

ADSVF characterization

The amount of adipose tissue harvested was 181.3 ml (S.D. 50.8). The number of total viable nucleated cells ($\times 10^6$) was 50.5 (S.D. 23.8, range 16.7–92.6) with 88.0% (S.D. 4.8) cell viability. Cellular components inside the ADSVF were identified by flow cytometry analysis in accordance with International recommendations [7]. The surface markers CD45, CD34, CD90, CD146 and CD14 were used in combination with DRAQ5 and DAPI to exclude debris, red blood cells and dead cells. The frequency of mesenchymal-like stem cells was estimated using the colony-forming unit fibroblastic (CFU-F) clonogenic assay. Patients received 3.76 (S.D. 1.85) $\times 10^6$ viable cells into each finger. The infused cells contained 3.7% (S.D. 1.9) CFU-F. Cellular composition included 49.1% (S.D. 18.0) leucocytes ($CD45^+CD34^-$), 9.8% (S.D. 8.8) endothelial cells ($CD34^+CD146^+CD45^-$), 36.0% (S.D. 14.5) stromal cells ($CD45^-CD34^+CD146^-CD90^+$) and 5.1% (S.D. 2.3) macrophages ($CD45^+CD14^+CD34^{dim}$).

Assessment of safety and efficacy

Adverse events and efficacy endpoints were assessed by a single evaluator at 12 months following ADSVF injection. These included the CHFS questionnaire, Kapandji score assessing opposition of the thumb, grip and pinch strength using dynamometers, lateral range of motion of the fingers assessed by measuring the distance between the thumb and index finger (first corner) and the sum of

the distances between the four fingers (second, third and fourth corners) upon maximum stretch.

RP severity relied on the Raynaud's Condition Score (RCS). Patients had to record the frequency, duration and severity of attacks in the previous week [8] on a scale from 0 to 10. Hand pain was evaluated with a visual analogue scale (VAS, 0–100). The outcomes of DUs were recorded. The VSS (0–3) was obtained by nail-fold capillary microscopy. The modified Rodnan skin score (mRSS) applied to the hands (0–18) evaluated skin thickening on the dorsal hand and the first and second phalanges of the most affected finger. Sclerodactyly was assessed by the circumference of the fingers measured with a calibrated jeweller's ring. The overall disability was assessed by the Scleroderma Health Assessment Questionnaire (SHAQ).

In addition, at the 12 month follow-up, the benefit of the procedure was assessed by the patients using a self-completed questionnaire specifically designed for the study. Patients were asked to estimate the changes in health status, well-being, social relationships and psychological state since the ADSVF injection. They estimated the benefit on a VAS or on a scale from much better to worse.

Data analysis

Continuous data are summarized as mean (S.D., range minimum–maximum). Mean changes from baseline were analysed using the paired Student's *t*-test at a $P < 0.05$ level. Spearman's correlation was used to analyse the relationship between biological parameters and clinical parameters.

Results

Twelve females were assessed 1 year after the ADSVF injection, by the same practitioners. No adverse events occurred between the 6 and 12 month follow-ups (in hands and liposuction areas). The main results are detailed in Table 1. The baseline–12 months comparison showed a significant improvement of 46.8% for the SHAQ ($P = 0.001$), of 51.3% for the CHFS score (Fig. 1) and of 63.2% for the RCS ($P < 0.001$ for both). The RCS was of 7.2 (S.D. 0.9) at baseline and decreased to 2.9 (S.D. 1.4) at 6 months ($P = 0.011$) and to 2.5 (S.D. 1.4) at 12 months ($P < 0.001$). The VAS for pain remained lower at 12 months compared with the baseline and approached statistical significance ($P = 0.052$, Table 1); however, the VAS for pain had increased from 6 months. Five patients had active DUs at inclusion. Two of the five patients with persisting DUs at the 6 month follow-up had healed ulcers at the 12 month follow-up. Three patients had persisting DUs. Six of the seven patients without DUs at entry did not develop DUs. One patient developed two DUs at the 12-month follow-up. A decrease in the total number of DUs was noted from 15 at entry to 9 at 12 months. None of the eight patients with previous iloprost infusion required a new infusion.

Forearm and hand grip strength improved, with a mean for both hands of 15.4 kg (S.D. 5.7) at baseline *vs* 20.1 (6.9) at 12 months ($P = 0.002$). Lateral pinch strength also increased, from 2.9 kg (S.D. 2.1) to 4.9 (1.8) ($P = 0.028$). An improvement of the first corner distance was noted from 110.7 mm (S.D. 23.9) to 128 (24.9) ($P < 0.001$). The sum of the second to fourth corner distances increased from 132.1 mm (S.D. 24.6) to 139.5 (28.3) ($P = 0.048$) in the non-dominant hand, whereas it did not improve in the dominant hand ($P = 0.059$). The Kapandji score improved ($P = 0.008$). The mean circumference of all fingers significantly decreased [61.3 (S.D. 2.2) *vs* 59.0 (2.0), $P = 0.001$]. Moreover, the mRSS focused on the hands significantly decreased [10.9 (S.D. 4.9) to 8.4 (5.5), $P = 0.014$].

The mean VSS for both hands significantly decreased, from 1.7 (S.D. 0.7) to 1.1 (0.7) ($P < 0.001$). Except for the sum of the second to fourth corner distances, both hands showed significant results (Table 1). Nine patients (75%) reported faster growth of their nails and 10 patients (83.3%) felt that their nails were of better quality. No correlation was observed between the characteristics of the injected ADSVF (number, viability, subpopulation of cells, CFU-F) and clinical outcomes.

All 12 patients completed the self-completed questionnaire. Four patients indicated that they were extremely satisfied with the procedure, five indicated satisfaction and three indicated moderate satisfaction. The patients were asked the following question: what do you think about your health today compared with the previous year? Two patients stated that it was much better, seven that it was better and three said it was identical. Eight patients said they were more enthusiastic, seven more energetic and six happier. Moreover, none reported to be sadder, more exhausted or more tired. The impact of the disease on their family life was reduced in one-third of patients. An increase in the patient's participation in housework [from 48.1 (S.D. 20.6) to 65.9 (22.1), $P = 0.05$] and in social activities [from 59.7 (S.D. 14.7) to 71.6 (16.9), $P = 0.06$] was observed. Emotional relationship involvement improved slightly [from 56.2 (S.D. 23.6) to 67.3 (25.4), $P = 0.16$]. Finally, regarding various types of daily activities, no patient felt more disabled and half were improved.

Discussion

The 12 month assessment showed encouraging long-term results of a single s.c. injection of ADSVF on hand disability, strength and motion; RP severity; DUs; VSS and sclerodactyly and consequently quality of life. All efficacy parameters that were significant at the 6 month follow-up remained improved when evaluated at 12 months, except for hand pain. It should be noted that some scores (Kapandji, mRSS applied to the hands) that were not significant at 6 months became significant at 12 months. This suggests a possible continuous and progressive effect of the cell-based therapy on skin fibrosis.

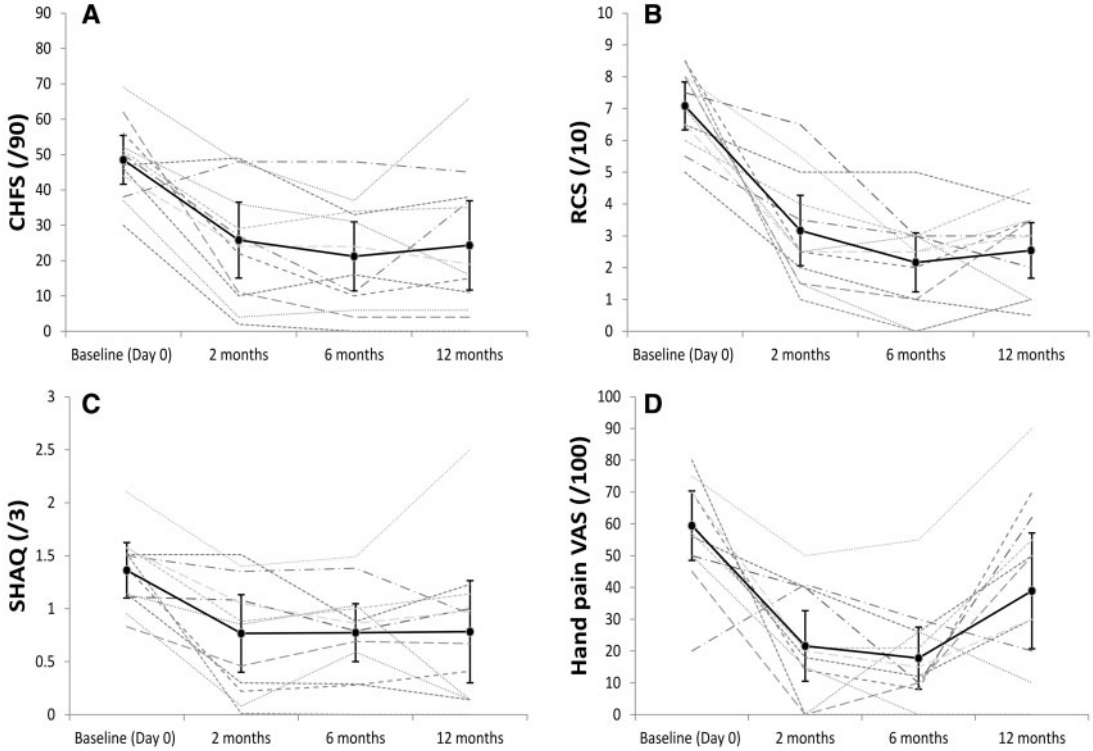
Thus the ADSVF can counterbalance various pathological mechanisms involved in hand damage, i.e. ischaemia and skin fibrosis. Due to its pleiotropic effects, ADSVF seems to be a promising therapy for the hands of scleroderma patients. As an open-label study, these results

Table 1 Main results and outcomes from baseline to 6 and 12 months

	Baseline	6 months	Change (6 months–baseline)	*P*-value[a]	12 months	Change (12 months–baseline)	*P*-value[b]
SHAQ score (0–3)							
Mean (S.D.)	1.4 (0.3)	0.8 (0.4)	−0.6 (0.4)	0.001	0.8 (0.7)	−0.6 (0.5)	0.002
[min–max]	[0.8–2.1]	[0.0–1.5]	[−1.5 to −0.1]		[0.0–2.5]	[−1.5 to −0.4]	
CHFS total (0–90)							
Mean (S.D.)	48.5 (10.8)	21.2 (15.4)	−27.3 (17.2)	0.002	24.3 (19.8)	−24.2 (18.1)	<0.001
[min–max]	[30.0–69.0]	[0.0–48.0]	[−58.0–10.0]		[0.0–66.0]	[−58.0–7.0]	
RCS (0–10)							
Mean (S.D.)	7.2 (0.9)	2.9 (1.4)	−4.3 (2.1)	0.011	2.5 (1.4)	−4.5 (1.8)	<0.001
[min–max]	[6.5–8.0]	[2.5–3.0]	[−7.0 to −1.5]		[0.5–4.5]	[−7.5 to −1.5]	
Hand pain VAS (0–100)							
Mean (S.D.)	59.4 (17.2)	17.8 (15.3)	−41.7 (22.7)	<0.001	38.9 (28.6)	−20.5 (32.6)	0.052
[min–max]	[50.0–72.5]	[9.0–26.0]	[−80.0–10.0]		[0.0–90.0]	[−80.0–15.0]	
Jamar score, kg							
Dominant hand, mean (S.D.)	16.0 (5.8)	19.4 (7.4)	4.8 (6.4)	0.033	20.9 (6.4)	5.8 (5.1)	0.004
[min–max]	[9.0–26.5]	[5.0–30.0]	[−6.0–17.0]		[12.0–30.0]	[0.0–17.0]	
Non-dominant hand, mean (S.D.)	14.9 (6.1)	17.6 (8.0)	4.0 (3.5)	0.002	19.3 (7.8)	5.1 (4.2)	0.002
[min–max]	[6.0–26.0]	[3.5–29.0]	[0.0–13.0]		[8.0–30.0]	[0.0–14.0]	
Pinch score, kg							
Dominant hand, mean (S.D.)	1.3 (1.1)	2.3 (1.3)	1.0 (1.1)	0.009	5.1 (2.0)	2.1 (2.9)	0.038
[min–max]	[0.2–4.1]	[0.9–5.4]	[−1.1–3.4]		[2.0–9.0]	[−2.5–7.5]	
Non-dominant hand, mean (S.D.)	1.3 (0.9)	2.1 (1.0)	0.8 (1.2)	0.050	4.8 (1.6)	1.8 (2.4)	0.030
[min–max]	[0.2–3.2]	[0.7–3.6]	[−1.1–3.4]		[2.0–7.5]	[−2.5–7.0]	
Mean circumference of fingers (ring size)							
Dominant hand, mean (S.D.)	61.9 (2.2)	59.8 (2.4)	−2.1 (1.1)	<0.001	59.8 (2.1)	−2.2 (2.3)	0.007
[min–max]	[58.8–66.0]	[56.0–64.4]	[−4.6 to −0.4]		[56.2–64]	[−7.6–1.6]	
Non-dominant hand, mean (S.D.)	60.7 (2.3)	58.1 (2.2)	−2.5 (1.5)	<0.001	58.2 (1.9)	−2.5 (1.6)	<0.001
[min–max]	[57.0–64.2]	[54.0–62.8]	[−4.8–0.4]		[55.4–61.6]	[−6.0 to −0.4]	
VSS (0–3)							
Dominant hand, mean (S.D.)	1.7 (0.8)	1.5 (0.7)	−0.2 (0.3)	0.010	1.1 (0.7)	−0.6 (0.4)	<0.001
[min–max]	[1.0–2.3]	[0.9–2.0]	[−0.4–0.0]		[0.0–2.4]	[−1.4–0.0]	
Non-dominant hand, mean (S.D.)	1.6 (0.7)	1.3 (0.7)	−0.3 (0.3)	0.003	1.1 (0.7)	−0.6 (0.5)	0.002
[min–max]	[0.9–2.2]	[0.8–2.0]	[−0.8–0.2]		[0.0–2.0]	[−1.6–0.0]	
mRSS applied to hand (0–18)							
Mean (S.D.)	10.9 (4.9)	9.9 (6.0)	−1.0 (2.8)	0.246	8.4 (5.5)	−2.5 (3.0)	0.014
[min–max]	[3.0–18.0]	[1.0–18.0]	[−5.0–4.0]		[1.0–18.0]	[−7.0–3.0]	

[a]*P*-value from baseline to 6 months. [b]*P*-value from baseline to 12 months. *P*-values were determined by paired Student's *t*-test. CHFS: Cochin Hand Function Scale; mRSS: modified Rodnan skin score; RCS: Raynaud's condition score; SHAQ: Scleroderma Health Assessment Questionnaire; VAS: visual analogue scale; VSS: vascular suppression score (0 = no avascular area, 1 = one or two discrete avascular areas, 2 = more than two avascular areas, 3 = extensive and confluent avascular areas).

Fig. 1 Twelve-month follow-up for SSc patients receiving ADSVF injection



(**A**–**D**) Individuals (grey dotted lines) and average evolution (with error bar corresponding to the 95% CIs of means) (black line) of the (**A**) CHFS, (**B**) RCS, (**C**) SHAQ and (**D**) hand pain VAS. ADSVF: autologous adipose-derived stromal vascular fraction; CHFS: Cochin Hand Function Scale; RCS: Raynaud's condition score; SHAQ: Scleroderma Health Assessment Questionnaire; VAS: visual analogue scale.

should be interpreted with caution and should be confirmed by a randomized placebo-controlled trial in a larger population. The present data did not allow us to attribute the clinical benefit to a specific subpopulation of cells within the ADSVF. Additional phenotypic and functional characterizations of the ADSVF will help us to better understand the mechanisms supporting its the clinical effects.

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