Document section:

Full study protocol and statistical analysis (2020.05.28)

NCT03060551

Clinical efficacy and safety of injection of stromal vascular fraction derived from autologous adipose tissues in systemic sclerosis patients with hand disability: a phase I proof-of-concept trial

1. Study patients and progress

All candidates were regarded as eligible if they were above 19 years old, and fulfilled the 2013 classification criteria for SSc [1]. Patients with uncontrolled infection in digital ulcer or significant organ involvement needing intensive immunosuppressive treatment within 6 months before enrollment were excluded. Between July 2018 and December 2019, a total of twenty subjects were enrolled in this single center, open label, phase I trial. Informed consent was obtained from all participants according to the principles of the Declaration of Helsinki. This trial was approved by the Institutional Review Board of Seoul St. Mary's Hospital of the Catholic University of Korea (approval number: KC16CISF0365). Among the initially enrolled patients, one patient refused further procedures after baseline evaluation, while another patient dropped out due to complications during local anesthesia right before SVF injection, which is described in detail in the results section. Finally, eighteen subjects received SVF treatment, and were fully followed-up for the whole scheduled period for clinical assessment. SVF extraction and injection were performed in outpatient setting within at least 1 month after baseline evaluation. All patients were regularly assessed for intervention-related adverse events and clinical efficacies at 2 weeks (2W), 6 weeks (6W), 12 weeks (12W), and 24 weeks (24W) after their procedure.

2. Manufacture and administration of SVF

Fat tissue was harvested from the abdomen as the first choice, and the thigh was selected as the second choice, in the case where the amount of the fat obtained from the abdomen was insufficient. Whole harvest procedures were performed under local anesthesia, and by a suction-assisted Coleman method after tumescent solution infiltration (Figure 1A). If possible, we tried to harvest about 100 mL of fat tissue after excluding the tumescent volume, in order to obtain more cells for injection. A closed system kit (SmartX® kit; DongKoo Bio & Pharma Co., Ltd., Seoul, Korea) was used to extract SVF from harvested adipose tissue. This process was performed within 50 minutes through repeated process of collagenase mixing, centrifuge, washing and filtering in the closed kit system. The obtained SVF, including heterogenous cells and soluble factors, was diluted to 7.0 mL, by mixing with a normal saline solution. Then 6 mL of the acquired and diluted 7 mL solution was divided into 6 syringes for injection (Figure 1B). About 15 minutes before the termination of SVF isolation, the fingers were blocked using 1% lidocaine by digital nerve block method. A total 6 mL of SVF was injected into the 10 fingers of each patient, in the manner of 0.6 mL for each finger. Injection sites were

located at the proximal interphalangeal joint, distal interphalangeal joint, and distal palmar crease for the long fingers, and the 3 points dividing from the metacarpophalangeal joint to the tip of the finger for the thumb (Figure 1C). Injection was performed by a retro-tracing technique, without puncturing the vessel. After gentle compression and cleansing with saline solution of the injection sites, the patient was discharged without dressing. After 20 minutes of treatment, hands were allowed to be used freely, and washing was also allowed. Liposuction lesions were dressed every 2–3 days, and sutures were removed 2 weeks after surgery. The remaining 1 mL was kept for biological laboratory study for cell count. Not only mesenchymal cells, but numerous cells, including red blood cell, endothelial cell, hematopoietic stem cell, and adipocyte, are presented in 1mL of SVF. Therefore, in order to obtain accurate mesenchymal stem cell count results, the number of cell count was performed after SVF was applied to the plate for 12 h.

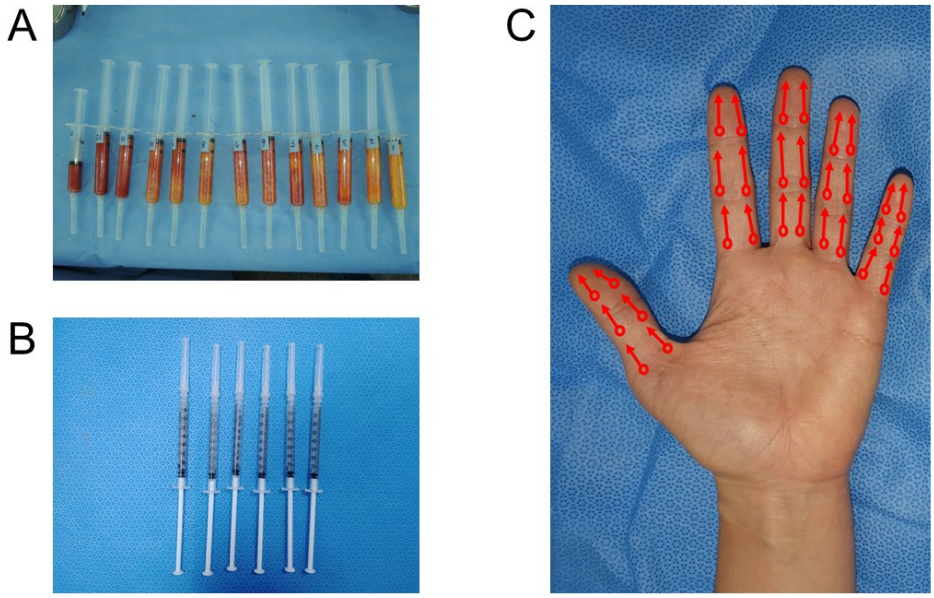

Figure 1. Preparation and injection of autologous fat tissue-derived stromal vascular fraction. (**a**) harvested autologous adipose tissues. (**b**) prepared stromal vascular fraction (SVF) mixtures for injection after the refinement. (**c**) Injection sites and needling directions of SVF mixtures. Circles indicate entry points of SVF injections, and arrows indicate directions of inserted needles using retro-tracing technique.

3. Outcomes related to skin fibrosis, hand functions, QOL, and digital ulcers

The modified Rodnan skin score (mRSS, ranging 0 to 51, with higher scores representing more severe skin fibrosis) was applied to assess the severity of overall skin fibrosis [2]. In order to evaluate the degree of hand fibrosis in particular, mRSS for hands (ranging 0 to 12) was defined as the sums of scores of mRSS in both hands and fingers. The finger circumferences of the second to the fifth fingers measured in the middle portion of each proximal phalanx were used to assess the degree of hand edema. The Cochin hand function scale (CHFS, ranging 0 to 90, with higher scores representing more deteriorated hand function) scores, and the Kapandji score (ranging 0 to 10), which are both validated measures in past studies as outcomes for hand disability, were introduced in the present trial [3,4]. The severity of Raynaud's phenomenon and hand pain was assessed using the Raynaud's condition scale (ranging 0 to 10, with higher scores representing more severe Raynaud's phenomenon), and the hand visual analog scale (ranging 0 to 10, with higher scores representing more severe pain), respectively [5]. Disease-related QOL was measured by health assessment questionnaire (HAQ, ranging 0 to 3, with higher scores meaning lower QOL), EuroQol-5 dimensions time trade-off (EQ-5D TTO, ranging 0 to 1, with higher values representing better QOL) values, and EuroQol visual analog scale (EQ VAS, ranging 0 to 100, with higher scores representing better QOL) [4,6]. Digital ulcers, defined as lesions of at least 5 mm of diameter, and visible skin defect were evaluated in each finger of enrolled patients at baseline evaluation, and were regarded as healed when full epithelization occurred at any time of each follow-up, and persisted at the last follow-up visit.

4. Scoring of nailfold capillary microscopic findings

In order to assess the severity of microangiopathies in both fingers, a semiquantitative scoring system for nailfold capillary microscopic findings was adopted [18]. A total of six parameters (irregularly enlarged capillaries, giant capillaries, hemorrhages, loss of capillaries, disorganization of the vascular array, and capillary ramifications) were measured using the semiquantitative manner on the scale ranging 0 to 3, indicating more severe as scores were higher. Each parameter was scored for four fingers of both hands, except thumbs, and the mean score value of each parameter in both hands was finally presented.

5. Statistical analysis

Because most efficacy data consisted of non-parametric variables, results were shown as median values and interquartile range. The Wilcoxon signed-rank test was performed

to analyze differences between baseline and at 2W, 6W, 12W, and 24W, respectively, using IBM-SPSS Statistics version 24.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was considered at $p < 0.05$. Safety profiles were descriptively analyzed.

6. References

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