Long-term efficacy and safety of regenerative treatment for male stress urinary incontinence using autologous adipose-derived regenerative cells

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Running title: SUI regenerative therapy with ADRCs

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Abstract

Objectives

To investigate long-term efficacy and safety of periurethral injection of autologous ADRCs for treatment of post-prostatectomy stress urinary incontinence.

Methods

Thirteen patients with persistent stress urinary incontinence after prostate surgery (radical prostatectomy, 10 patients; holmium laser enucleation of the prostate, 3 patients) underwent periurethral injection of ADRCs and were followed up for more than 4 years. A 24-hour pad test was performed consecutively for 4 days in each evaluation period, and changes in the mean daily leakage volume during the 4 days from baseline to 60 months after treatment were evaluated.

Results

Mean follow-up period was 69 months (55 to 72 months). Mean leakage volume/24 hours in all patients changed from 260.7 to 152.7 g. Urinary incontinence progressively improved up to 12 months after treatment in 10 patients, who maintained improvement up to the final assessment, with the mean daily leakage volume decreasing from 281.5 to 119.0 g (reduction rate: 57.7%). The other 3 patients showed no improvement at 1 year and at the final assessment. After the perioperative period, significant adverse

events or prostate-specific antigen increase was not observed during long-term follow-up.

Conclusion

The present study showed that periurethral injection of autologous ADRCs is a safe and feasible treatment modality with long-term efficacy for patients with male stress urinary incontinence caused by urethral sphincter deficiency.

Key words: adipose-derived regenerative cells, cell therapy, male, prostatectomy, stress urinary incontinence,

Abbreviations & Acronyms

ADRCs = adipose-derived regenerative cells

SUI = stress urinary incontinence

PMDA = Pharmaceuticals and Medical Devices Agency

Introduction

The prevalence of SUI is relatively low in men as compared with women, but an epidemiologic investigation for lower urinary tract symptoms in Japan reported that 820,000 men are suffering from SUI[1]. A common cause of SUI in men is sphincter dysfunction following prostatectomy for cancer and benign prostatic hyperplasia. The number of prostatectomies for cancer has been increasing according to the increased prevalence of prostate cancer due to rapid population aging and widespread prostate-specific antigen screening. Despite the introduction of robotic radical prostatectomy, 20 to 30% of patients have SUI persisting longer than 1 year [2, 3]. Pelvic floor muscle training is the most widely recommended first-line treatment for early incontinence that follows prostatectomy within the first 6 to 12 months. Some studies reported earlier recovery of post-operative incontinence by the pelvic floor muscle training [4]. However, the effectiveness of the treatment is limited and there is no evidence-based data for the recommendation because existing trials were mostly neither randomized nor controlled [4]. Although artificial urethral sphincter implantation is a standard treatment for severe male SUI, there is a lack of less invasive effective treatment for mild to moderate male SUI.

We developed a novel treatment strategy to regenerate urethral sphincter function,

using autologous ADRCs without the need of cell culture and conducted a first-in-man study [5]. ADRCs consisted of heterogeneous cell populations including adipose stem cells isolated from the harvested adipose tissue by using the Celution™ system. We published the 1-year short-term results for 11 male patients with SUI [6]. Although favorable efficacy and safety were confirmed at 1 year after treatment, long-term efficacy and safety should be confirmed. In the present study, we assessed long-term durability of efficacy and safety in 13 male patients with SUI caused by urethral sphincter deficiency who underwent periurethral injection of ADRCs and were followed up for more than 4 years.

Materials and Methods

The present study was approved by the Ethics Committee of the Nagoya University Graduate School of Medicine (No.657-3) and the committee of the Japanese Ministry of Health, Labor and Welfare according to the Guidelines on Clinical Research using Human Stem Cells (No.0315-3), and written informed consent was obtained from the patients.

The detailed procedure was described in previous reports [5, 6], but an outline of the treatment strategy is shown in Fig. 1 and described briefly as follows. The autologous

tissue source was harvested from the abdomen by liposuction. ADRCs were isolated from the tissue without culture using the Celution^{TM} system (Cytori Therapeutics Inc., San Diego, CA, USA). Finally, ADRCs were transurethrally injected into the urethral sphincter. This treatment can be completed as a single surgical procedure within 3 to 4 hours.

Patients

Thirteen patients with mean age of 75 years (68-81 years) were treated and followed up for more than 4 years (Table 1). The causes of SUI were sphincter deficiency after radical prostatectomy in 10 patients and holmium laser enucleation of the prostate in 3 patients. The inclusion criteria for the patients with prostate cancer were persistence of SUI for more than 2 years after surgery, localized low-risk prostate cancer with a preoperative prostate-specific antigen level of <10 ng/mL and Gleason score of 6 points or less, negative surgical margin on pathological examination of the resected prostate specimen, and no evidence of recurrence or metastasis, with undetectable levels of prostate-specific antigen. All enrolled patients had persistent SUI in spite of post-operative pelvic floor training longer than 3 months.

Harvesting adipose tissue (liposuction)

Under general anesthesia, 250 mL of adipose tissue was harvested from the anterior abdominal wall by making 2 3-mm incisions. Ringer's lactate was first infused in the subcutaneous layer and the adipose tissue was harvested. The suctioned adipose tissue was placed in saline and allowed to stand to settle the blood and cellular debris; adipose tissue floated at the top of the mixture. Following to harvest of the adipose tissue for isolation of ADRCs, additional adipose tissue of over 20mL was obtained for preparing the mixture of intact adipose tissue and ADRCs for injection.

Isolation of ADRCs

ADRCs were isolated from the harvested adipose tissue using the Celution^{TM} system, which is a commercially available kit designed to isolate ADRCs from human adipose tissue in a short time. This instrument allows the isolation of therapeutic doses of autologous ADRCs after liposuction without the need for culture. The final concentrated cell output was measured using a NucleoCounter (Chemometec, Allerod, Denmark), which exclusively detected nucleated cells. By using the Celution^{TM} system, we could obtain a 5-mL solution containing concentrated ADRCs.

Periurethral injection of ADRCs

After liposuction and isolation of ADRCs, transurethral endoscopic injection of ADRCs was performed. For periurethral injection, 2 distinct formulations were produced: 1 mL of the isolated ADRC fraction alone was preserved for direct injection, and another 4 mL of the fraction was mixed with intact autologous adipose cells, yielding a total of 20 mL of this combined solution.

A 22-F rigid endoscope was used to inject the processed ADRC solution. Under endoscopic vision, a puncture needle was passed through the endoscope into the urethra at the region of the external urethral sphincter. The 18-G needle was 35 cm in length and graduated in centimeters, and was specially ordered. The ADRC solution was injected after puncturing the urethra at the region of the external urethral sphincter under endoscopic vision. Initially, a 1-mL solution was injected to a depth of 5 mm into the rhabdosphincter at 5 and 7 o'clock positions. Subsequently, 20 mL of the formulation containing ADRCs and adipose tissue was equally injected into the submucosal spaces at 4 and 8 o'clock positions to facilitate complete coaptation of the urethral mucosa. After the solution was injected, a 6-F urethral balloon catheter was placed and was removed the following day. The whole procedure from liposuction to injection of ADRCs is performed in a single procedure under general anesthesia.

Efficacy based on urine leakage volume

The amount of incontinence was evaluated with a 24-hour pad test, and the total daily leakage volume was calculated. The 24-hour pad test was performed consecutively for 4 days in each evaluation period (baseline, 2 weeks, 1, 3, 6, 9, 12, 15, 18, 24, 30, 36, 48 and 60 months). Changes in the mean daily leakage volume during the 4 days from baseline to 60 months after treatment were evaluated. We regarded the decrease of mean daily leakage volume during as SUI improvement in this analysis. In addition, we evaluated the numbers of patients who achieved decreased leakage volume of greater than 50% as compared with baseline, which is a standard definition of significant SUI improvement [7].

Safety

Any subjective adverse events were reported, and blood examination including prostate-specific antigen level was performed at each visit.

Statistical analyses

For each patient, the change and/or percent change in leakage volume/day at

baseline and at each visit were calculated. Since this was an exploratory study with a small sample size and the aim was to confirm the long-term durability of decrease in leakage volume, statistical analysis for efficacy was not included in the purpose of this report.

Results

Mean follow-up period was 69 months (55 to 72 months). Changes in mean leakage volume for each patient and all patients from baseline to the final assessment (4 or 5 years after treatment) are shown in Figure 2 and Figure 3, respectively. After injection, mean leakage volume/24 hours for all patients decreased from 260.7 to 152.7 g. Urinary incontinence progressively improved up to 12 months after treatment in 10 of the 13 patients, and 1 patient with moderate incontinence achieved total continence at 14 weeks after injection. In the 10 patients who showed improvement at the final assessment, the mean daily leakage volume improved from 281.5 to 119.0 g (reduction rate: 57.7%). In 3 other patients without improvement, mean daily leakage volume increased from 191.1 to 273.9 g. In patients with decreased leakage volume, the improvement was maintained during long-term follow-up of 4 to 5 years after treatment (Fig. 2, 3). At the final assessment, 5 of 10 patients with improvement achieved a

decrease in leakage volume of more than 50% (Fig. 4).

Six patients developed transient subcutaneous hemorrhage following liposuction that spontaneously disappeared within one month, and thereafter no subjective or objective adverse events were observed during the follow-up period. No patient with incontinence after radical prostatectomy had prostate-specific antigen recurrence.

Discussion

We conducted the first-in-man study of periurethral injection of ADRCs for post-prostatectomy SUI patients [5] and previously reported the 1-year outcome for the initial 11 patients [6]. In this exploratory and preliminary study, SUI improved progressively in 8 patients during the 1-year follow-up, as determined by a decrease in leakage volume in the 24-hour pad test, decreased frequency and amount of incontinence, and improved quality of life evaluated by a validated symptom/QOL questionnaire. One patient achieved total continence. Mean maximum urethral closing pressure and functional profile length demonstrated a significant increase after treatment. Magnetic resonance imaging showed the sustained presence of the injected adipose tissue, and enhanced ultrasonography demonstrated a progressive increase in blood flow to the injected area in all patients. Thereafter, a Korean group treated 6 male SUI

patients after radical prostatectomy following our procedure, and confirmed the improvement of subjective symptoms and quality of life along with improved urethral sphincter function during a 12-week follow-up [8].

Based on the outcomes of the preliminary clinical study, we started a multicenter, investigator-initiated clinical trial (ADRESU study), approved by the Japanese PMDA in September 2016 (UMIN-CTR: UMIN 000017901, Clinical Trials.gov: NCT02529865) [9] and have completed the enrolment of all 45 patients. We are aiming at approval of this regenerative treatment by the PMDA after 1 year of follow-up.

Although favorable efficacy and safety were confirmed at 1 year after treatment in the preceding clinical study, long-term efficacy and safety should be confirmed. In the present study, we reported the long-term efficacy and safety of this regenerative treatment in 13 patients, including the initial 11 cases. We confirmed that the improvement persisted up to 5 years in patients with initial improvement at 1 year, and that no long-term adverse effect or recurrence of prostate cancer occurred.

In the present study, 10 patients demonstrated the long-lasting improvement of incontinence at 4 to 5 years after treatment. Nine of these 10 patients showed an improvement at one year after treatment (reduction rate of leakage volume: 48.6%), which was maintained at the time of the final assessment. In particular, 6 of 9 patients

achieving the improvement at one year demonstrated the reduction of leakage volume by greater than 50 % (mean 62.4%) at this point. On the other hand, 3 patients without improvement at the long-term follow-up did not have reduction of the leakage volume at one year after treatment. The aim of the present report is to prove the long-term durability of this treatment, however, it can also be said that the improvement of leakage volume at one year strongly predict the long-lasting efficacy in future. Since it was difficult to define the ideal/suitable indications for this treatment in this explorative study, we are going to investigate it in previously mentioned on-going ADRESU study

Adipose tissue contains multipotent cells that are similar to mesenchymal stem cells, and the number of stem cells in adipose tissue is 100 times that in bone marrow. These adipose stem cells can differentiate into bone, cartilage, nerve, blood vessels, and muscle [10, 11, 12, 13]. Adipose stem cells also secrete a variety of cytokines, especially those associated with angiogenesis, such as vascular endothelial growth factor and hepatocyte growth factor [14, 15]. Unlike bone marrow cells, adipose tissue can be easily and safely harvested in large quantities with minimum morbidity. ADRCs isolated with the Celution™ system were composed of a heterogeneous cell population including adipose stem cells and were also reported to show pluripotency and secretion of cytokines [16]. Based on preclinical experimental injection of adipose stem cells or ADRCs into the rat

urethra, it was suggested that the mechanisms involved in the improvement of sphincter function included a bulking effect, regeneration of smooth muscle, and increased blood flow caused by adipose stem cells [17] or ADRC injection [18], as confirmed also by our previous clinical study [5, 6]. We are conducting further analysis of cell characteristics using human ADRCs isolated with the Celution™ system.

There are some limitations in the present study. This was a preliminary and exploratory study with a small sample size that could not be used to demonstrate statistical efficacy. An ongoing multicenter investigator-initiated clinical trial (ADRESU Study) will enable statistical assessment of this novel regenerative treatment for male SUI.

In conclusion, periurethral injection of autologous adipose-derived stem cells is a feasible treatment for male SUI caused by urethral sphincter deficiency. This novel treatment strategy is minimally invasive, can be performed within 3 hours in a single procedure, does not require ex-vivo culture using autologous stem cells, and can provide long-term efficacy.

Conflict of interest: None declared

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Legends to figures

Figure 1

An outline of the treatment strategy

Whole procedure can be completed as a single surgical procedure within 3 to 4 hours, without the need of ex-vivo cell culture. ADRCs: Adipose derived regenerative cells

Figure 2

Changes in mean daily leakage volume for 13 patients at each visit from baseline to final assessment

solid lines: patients with improvement in leakage volume, broken lines: patients without improvement

Figure 3

Changes in mean daily leakage volume of all patients, those with improved leakage volume and those without improvement, from baseline to final assessment.

Figure 4

Percent changes of daily leakage volume in all patients at the final assessment as compared with baseline

Table 1 Patients' characteristics, and outcomes on isolation of ADRCs

Patients	Age	Duration of SUI (months)	Type of prostate surgery	No. of isolated cells	% of viable cells
1	68	24	HoLEP (15g)	$7.5x10^6$	93.8
2	76	54	RP	$2.2x10^7$	91.2
3	85	104	RP	$2.2x10^7$	90.1
4	72	74	RP	$7.3x10^6$	91.4
5	78	78	RP	$2.4x10^7$	89.7
6	78	52	RP	$3.3x10^7$	90.2
7	71	29	RP	$7.5x10^6$	93.8
8	74	66	HoLEP (40g)	$2.5x10^6$	94.2
9	75	40	RP	$1.8x10^7$	90.0
10	79	61	RP	$2.3x10^7$	88.6
11	77	74	RP	$3.3x10^7$	90.9
12	70	24	RP	$7.0x10^7$	90.0
13	76	264	HoLep (38g)	$3.2x10^7$	90.6
Mean (Median)	75	72 (61)		2.5×10^7 (2.2×10^7)	91.1 (90.6)

HoLEP: holmium laser enucleation of the prostate (enucleation weight of prostate:gram), RP: radical prostatectomy

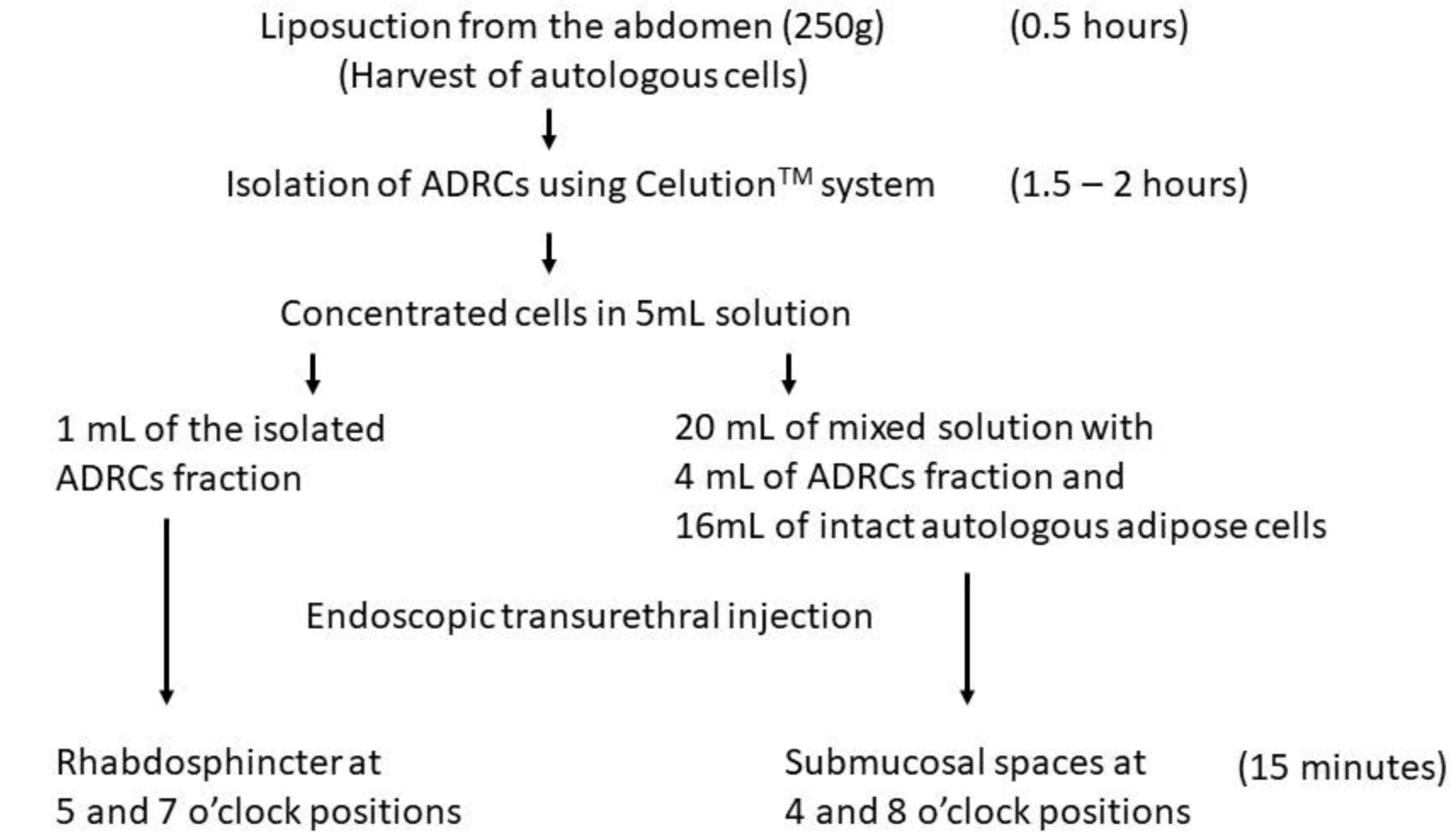
Liposuction from the abdomen (250g)
(Harvest of autologous cells)
(0.5 hours)
Isolation of ADRCs using Celution™ system
(1.5 – 2 hours)
Concentrated cells in 5mL solution
1 mL of the isolated
ADRCs fraction
20 mL of mixed solution with
4 mL of ADRCs fraction and
16mL of intact autologous adipose cells
Endoscopic transurethral injection
Rhabdosphincter at
5 and 7 o'clock positions
Submucosal spaces at
4 and 8 o'clock positions
(15 minutes)

Fig. 1

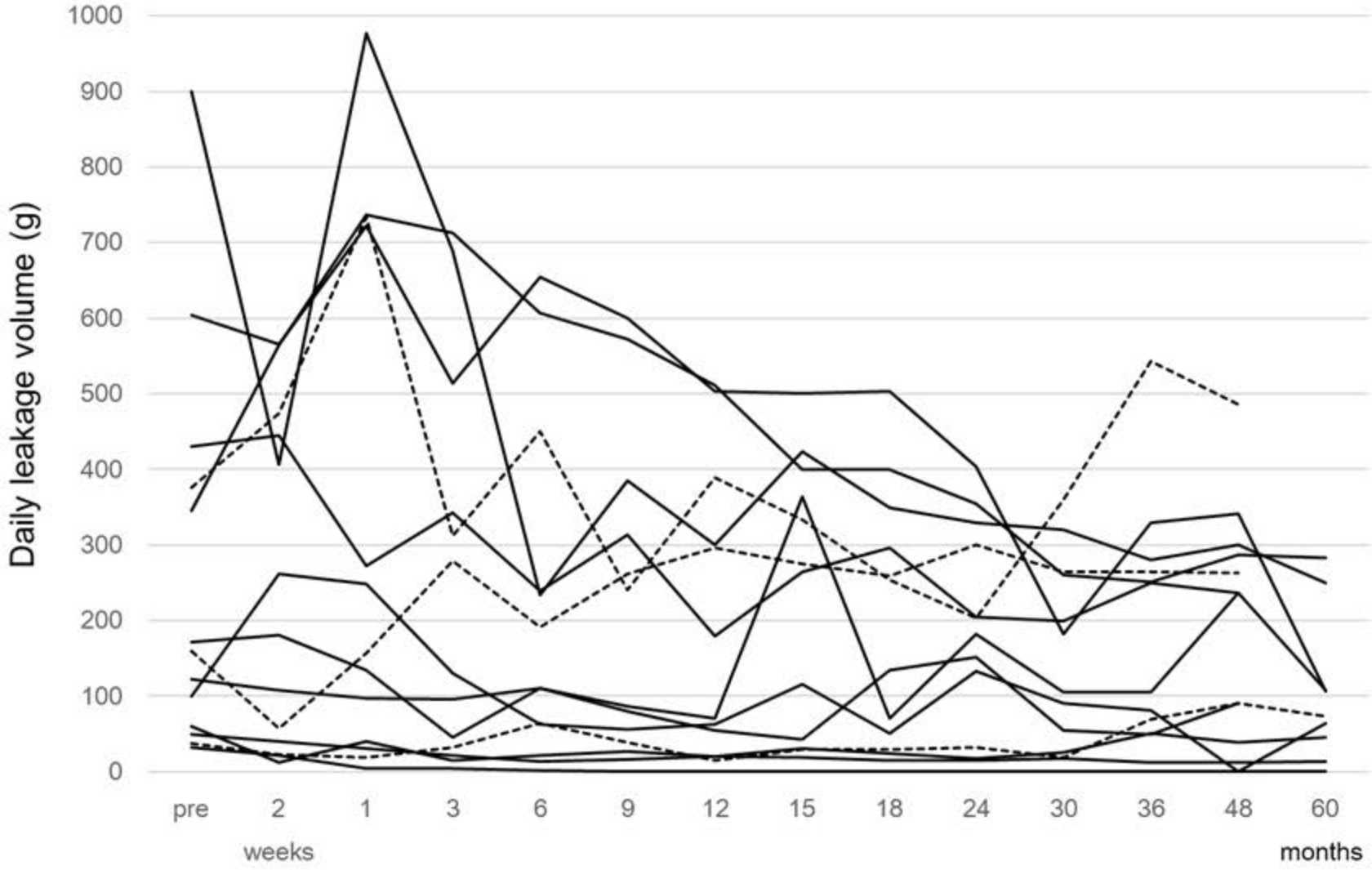
Daily leakage volume (g)
1000
900
800
700
600
500
400
300
200
100
0
pre
2
1
3
6
9
12
15
18
24
30
36
48
60
weeks
months

Fig. 2

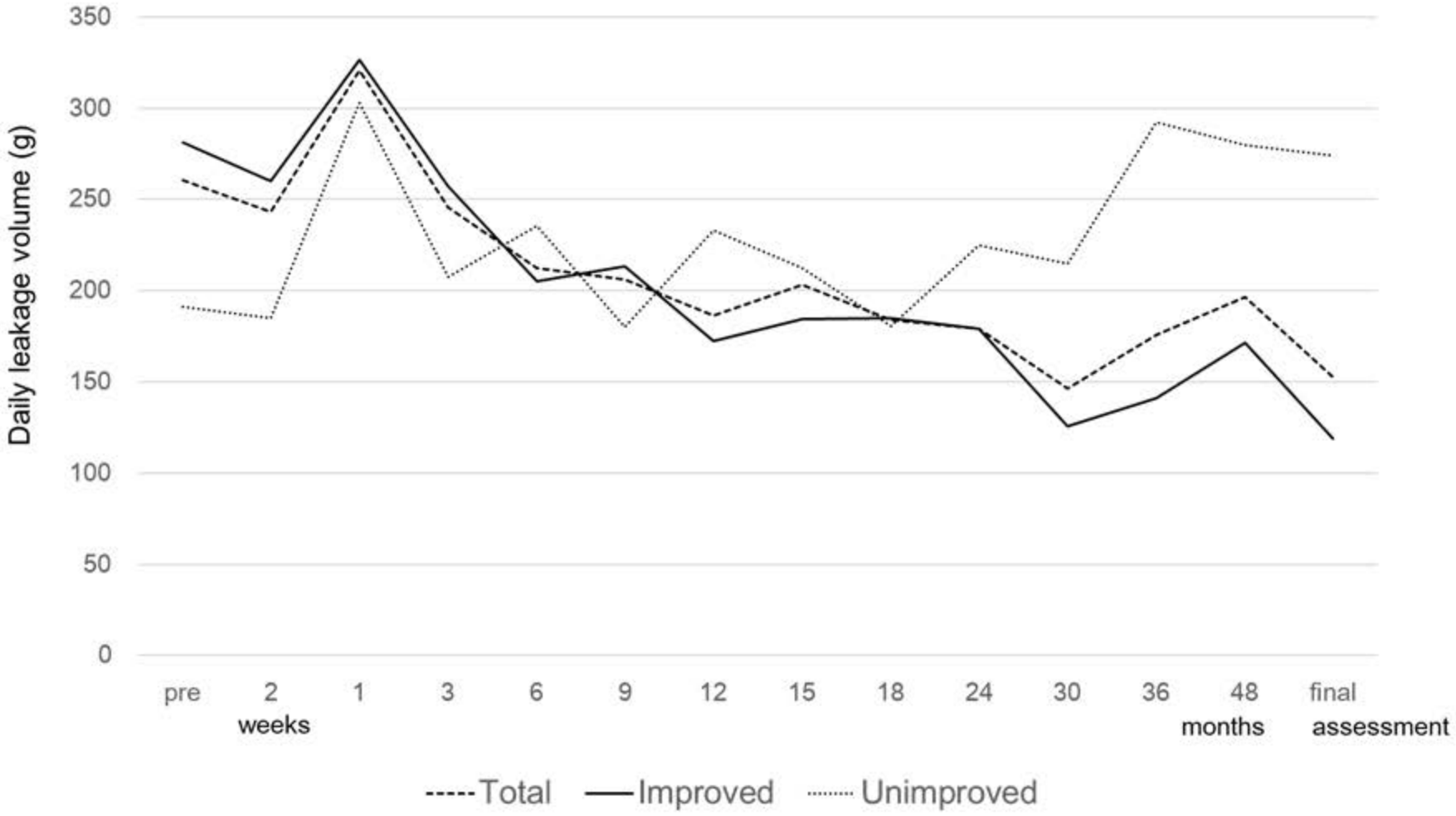
Daily leakage volume (g)
350
300
250
200
150
100
50
0
pre
2 weeks
1
3
6
9
12
15
18
24
30
36
48 months
final assessment
Total
Improved
Unimproved

Fig. 3

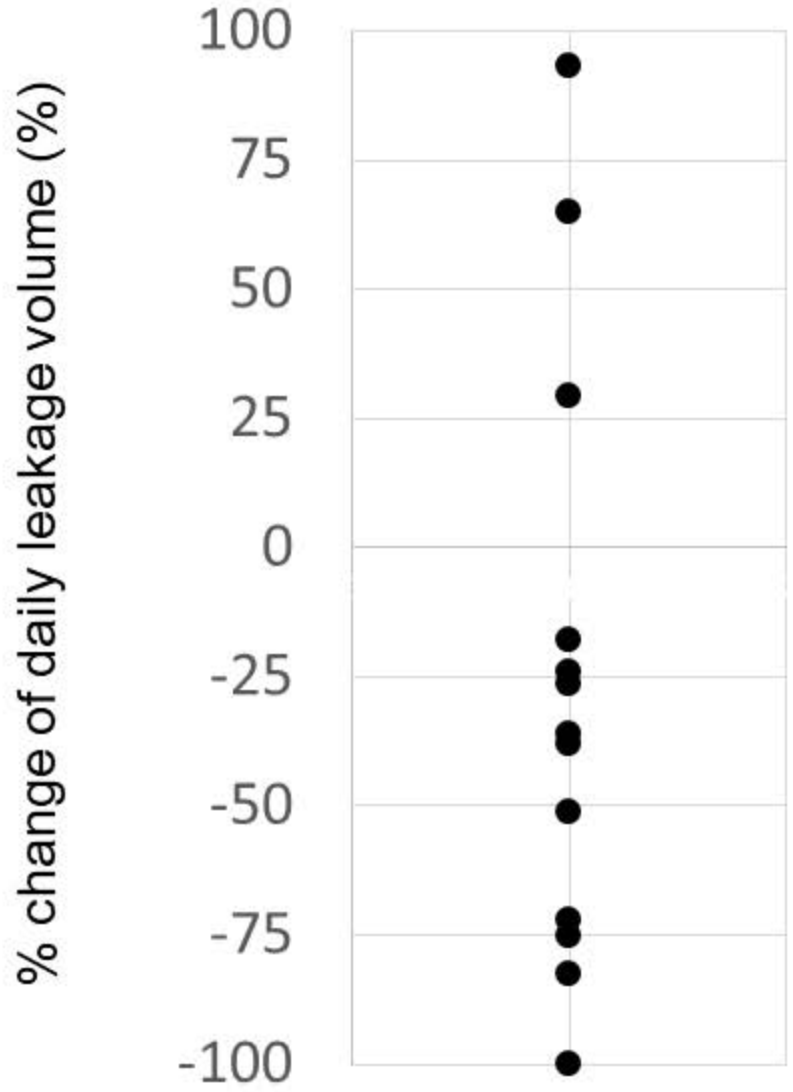

Fig. 4