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Adipose-derived regenerative cells and lipotransfer in alleviating breast cancer-related lymphedema: An open-label phase I trial with 4 years of follow-up

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Abstract

Patients with breast cancer-related lymphedema (BCRL) have reduced quality of life and arm function. Current treatments are palliative, and treatments improving lymphedema are lacking. Preclinical studies have suggested that adipose-derived regenerative cells (ADRCs) can alleviate lymphedema. We, therefore, aimed to assess whether ADRCs can alleviate lymphedema in clinical reality with long-term follow-up. We treated 10 patients with BCRL using ADRCs and a scar-releasing lipotransfer to the axillary region, and all patients were followed 1, 3, 6, 12, and 48 months after treatment. The primary endpoint was change in arm volume. Secondary endpoints were safety, change in lymphedema symptoms, quality of life, lymphedema-associated cellulitis, and conservative treatment use. There was no significant decrease in BCRL volume after treatment. However, self-reported upper extremity disability and arm heaviness and tension improved. Six patients reduced their use of conservative BCRL treatment. Five patients felt that their BCRL had improved substantially, and four of these would redo the treatment. We did not observe any cases of locoregional breast cancer recurrence. In this phase I study with 4 years of follow-up, axillary delivered ADRCs and lipotransfer were safe and feasible and improved BCRL symptoms and upper extremity function. Randomized controlled trials are needed to confirm the results of this study.

KEYWORDS

adipose tissue, fat graft, fat transfer, pilot study, stromal vascular fraction

1 | INTRODUCTION

Breast cancer-related lymphedema (BCRL) is a frequent and feared sequela of breast cancer treatment with axillary lymph node involvement.[1] Conservative lymphedema treatment with complete decongestive therapy and compression is the primary treatment; however, it requires lifelong compliance, which is time-consuming and costly. Previous preclinical studies have so far shown promising

potential for alleviation of BCRL using regenerative cell treatments.[2] Using adipose tissue as the source of autologous mesenchymal regenerative cells for treating BCRL is appealing, as it is obtainable in large quantities with minimal discomfort and allows for a safe, minimally invasive surgical procedure.

We have sought to alleviate BCRL using adipose-derived regenerative cells (ADRCs) in conjunction with a scar-releasing lipotransfer to the axilla and have previously presented our results up to 1 year.[3,4] However, several years of follow-up are necessary to determine the long-term efficiency and oncological safety.[5] Thus, we now present our final study results with 4 years of follow-up.

Lessons learned

- Adipose-derived regenerative cells and lipotransfer were safe and feasible in the treatment of breast cancer-related lymphedema.
- The treatment alleviated lymphedema symptoms, and treatment effectiveness was sustained for up to 4 years after surgery.

Significance statement

The evidence from this long-term, open-label, phase I study implies that adipose-derived regenerative cells and lipotransfer are safe and feasible in the setting of previous breast cancer and can potentially alleviate lymphedema and cellulitis. Effectiveness was observed shortly after treatment and sustained for up to 4 years after treatment. These promising results will need to be tested in a randomized, controlled, and blinded study to further document clinical efficacy.

2 | MATERIAL AND METHODS

2.1 | Trial design and registration

We conducted a prospective open-label, single-arm, and single-center phase I study evaluating the safety and feasibility of ADRCs and lipotransfer for the treatment of BCRL.

We aimed to include 10 patients for this study, and the eligibility criteria for participation were as follows: age between 18 and 70 years, unilateral BCRL, International Society of Lymphology stage I or II,[6] a recurrence-free disease for a minimum of 1 year, circumference difference of either upper or lower arm of 2 cm between healthy and lymphedematous arm, American Society of Anesthesiologists physical status score 1 or 2, written informed consent, and the ability to understand the Danish language. The exclusion criteria were as follows: history of other cancer types, diabetes mellitus, psychiatric conditions that could interfere with participation, and tobacco use.

Patients were evaluated 1, 3, 6, and 12 months and 4 years after the ADRC and lipotransfer treatment. The primary endpoint was a change in arm volume. Secondary endpoints were a change in lymphedema symptoms, health-related quality of life, lymphedema-associated cellulitis, conservative treatment use, treatment satisfaction, and safety.

All patients gave written informed consent before participation, and the study was registered at ClinicalTrials.gov before the inclusion of the first patient (NCT02592213). The study was approved by the Regional Committees on Health Research Ethics for Southern Denmark (S-2015010) and registered with the Danish Data Protection Agency (2008-58-0035). The ADRC preparation and handling of human tissue and cells were done in an authorized tissue establishment at the Department of Clinical Biochemistry and Pharmacology at Odense University Hospital (Danish Health and Medicines Authority, authorization no. 29035).

2.2 | ADRC and lipotransfer treatment

We have previously described the experimental BCRL treatment in detail.[3] In brief, liposuction was performed under general anesthesia without local anesthetics, as the effect of these on ADRC viability is uncertain.[7] We used either the abdomen or thighs depending on adipose tissue availability and preference of the patient using water jet-assisted liposuction (Body-Jet, Human Med AG, Schwerin, Germany). The lipoaspirate was decantated for 15 minutes. We intended to harvest 300 mL of lipoaspirate for the scar-releasing lipotransfer and ADRC isolation. First, we performed axillary rigottomy by injecting 30 mL of lipoaspirate to the axilla using a sharp cannula in a fan-shaped pattern to release the scar tissue in multiple subcutaneous levels. Next, we transferred the remaining lipoaspirate to the authorized tissue establishment for ADRC isolation, and the patient was transferred to the patient ward. The authorized tissue establishment was located at the Department of Clinical Biochemistry and Pharmacology on the same floor and adjacent to the Department of Plastic Surgery at Odense University Hospital. Transfer time of the adipose tissue from the operating theater to the authorized tissue establishment was less than 5 minutes. The ADRCs were then isolated from the lipoaspirate using an automated processing Celution 800/CRS system (Lorem Cytori Therapeutics, San Diego, California) per the manufacturer's instructions. Then, we transferred the ADRC suspension to a 5-mL syringe, of which we saved 1 mL for ADRC characterization. The syringe containing the remaining 4 mL of ADRC suspension was transferred to the patient ward, which was also located less than 5 minutes' walking distance from the autorized tissue establishment. Finally, we injected the remaining 4 mL back to the patient at the patient ward. The isolated ADRCs were injected in eight predefined points in the axilla adjacent to the axillary scar, in the same area and depth where fat grafting was performed. For each injection,

0.5 mL of the suspension was injected using a 25-gauge cannula for a total of 4 mL.

2.3 | ADRC characterization

Total viable nucleated cell recovery and viability percentages were determined using the Nucleocounter NC100 (ChemoMetec, Allerod, Denmark). Cellular components were identified by flow cytometry analysis with a panel of cell surface markers (CD34, CD90, CD31, CD73, CD235a−CD45−CD31−CD34+, and CD235a−CD45−CD31+CD34+) in agreement with International Federation for Adipose Therapeutics and Science and the International Society for Cellular Therapy recommendations.[8]

2.4 | Lymphedema volume estimation

We calculated the volume of both the lymphedematous and healthy arm using multiple circumference measurements using a previously described method.[9] The circumference measurements were made at five points on each arm: wrist, largest point on the lower arm, elbow, middle of the upper arm, and proximal on the upper arm. The length between each point was measured, and we used the same lengths and measuring points at each follow-up. Based on these five measurements, we divided the arm into four segments and calculated the volume of each segment using the truncated cone formula:

$$V = \frac{h\left(C_1^2 + C_1C_2 + C_2^2\right)}{12\pi},$$

where V is the segment volume, h is the length of the segment, and C_1 and C_2 are the two circumference measurements at the two ends of the segment. The total arm volume of both arms was calculated as the sum of the four segmental volumes.

The lymphedema volume was defined as the affected arm's volume compared with the healthy arm by subtracting the volume of the unaffected arm from the affected arm. For patients who wore compression garments, compression garments were removed only immediately before and during measurements.

We calculated each patient's body mass index (BMI) before and after treatment, in the case of weight fluctuations during the study, which may influence arm size. Before treatment, we recorded the patient's height and weight, and at the final follow-up, patients were weighed, and the same prerecorded height was used to calculate BMI.

2.5 | Lymphedema symptoms

We asked patients to rate their feeling of heaviness and tension in the lymphedematous arm on numerical rating scales ranging from 0 to 10, with 0 meaning no heaviness/tension at all and 10 signifying the worst heaviness/tension imaginable.[10]

2.6 | Patient-reported outcome questionnaires

We asked patients to fill out two patient-reported outcome questionnaires to assess HRQoL.

1. A quality of life measure for lymphoedema (LYMQOL).[11] The questionnaire is a 28-item questionnaire categorized into four domains: symptoms, appearance, function, and mood. Each item is then scored on a 4-grade Likert scale from 1 to 4. A score of 1 means that the patient is not bothered at all, and a score of 4 means the patient is bothered a lot. The last item in the LYMQOL is an overall quality of life numeric rating scale that is rated from 0 to 10, where 0 denotes poor quality of life and 10 denotes excellent quality of life.
2. The Disabilities of the Arm, Shoulder, and Hand (DASH) questionnaire[12] is a Danish-translated and validated generic upper extremity functional questionnaire consisting of 30 items evaluating upper limb-related activities and symptoms. Each item is scored on a 4-grade Likert scale from 0 to 4, which in turn is transformed into a 0 to 100 scale for disability of the hand, arm, and shoulder function or symptoms. A score of 0 means that the patient is not bothered at all, and a score of 100 means the patient is bothered a lot.

2.7 | Cellulitis

We asked all patients if they had been treated for cellulitis in their lymphedematous arm since their lymphedema diagnosis. The yearly incidence of cellulitis before treatment was calculated by dividing the total number of cellulitis incidents before treatment by the duration of lymphedema in years.

The yearly incidence of cellulitis after treatment was calculated by dividing the total number of cellulitis incidents after treatment by the individual follow-up time after treatment. No patients received any postoperative prophylactic antibiotics or other preventive treatments to minimize the risk of infection after treatment.

2.8 | Conservative lymphedema treatment

We asked patients whether they used a compression sleeve, compression gauntlet, night compression, or pneumatic compression device to treat their lymphedema. We further asked how frequently (on average) they used each treatment: daily, >3 days a week, 1-3 days a week, or <1 day a week. The use of conservative lymphedema treatment was compared before and after treatment.

2.9 | Treatment satisfaction

At the final follow-up, we asked all patients two standardized questions indicating their overall treatment satisfaction with treatment. The first question aimed to assess the overall satisfaction with the

outcome. It was phrased, "Concerning the experimental lymphedema treatment, do you feel that your lymphedema has improved significantly, from before treatment and up to now?" and we requested a "yes" or "no" response. For patients who answered "yes" we further asked the patients to describe what they felt had improved the most. The second question aimed to assess the significance of outcome improvement weighted against discomfort related to treatment and was phrased, "Concerning the experimental lymphedema treatment, knowing the course of treatment, your outcome and any discomfort you may have experienced, would you go through the same procedure again?"

2.10 | Long-term evaluation of adverse events

At each follow-up time, short-term adverse events were recorded by inspection of the injection and donor site using a prespecified form.[3] Furthermore, we asked an open-ended question: "Did you experience any discomfort related to the operation since your last visit?" To assess oncological safety at the final follow-up, we asked about new breast cancer diagnosis or recurrence along with other newly diagnosed diseases, changes in medication, and surgeries since their last visit.

2.11 | Statistical analyses

Continuous parametric data were described as means ± SD, and nonparametric data were described by median (interquartile range [IQR] and range). Patient-reported outcomes were analyzed with Friedman's test for multiple nonparametric comparisons and Dunn's post hoc test for multiple comparisons. Volumetric changes were analyzed by one-way analysis of variance with Dunnett's post hoc test for multiple comparisons. Cellulitis incidences per year was compared before and after treatment using the Wilcoxon rank-test. A two-tailed *P* value of less than .05 was considered statistically significant. Statistical analyses were performed using STATA 15 (StataCorp LP, College Station, Texas) and GraphPad Prism (version 8.00 for Windows, GraphPad Software, La Jolla, California) and conducted with a two-tailed significance level of .05 and reported with 95% confidence interval when applicable.

3 | RESULTS

We included 10 patients with BCRL in this open-label, single-arm, phase I trial. We screened 34 patients for inclusion and treated 11 patients, of whom 10 were included in this study (Figure 1 shows the study flowchart). One patient (no. 2) was immediately excluded after ADRC treatment because of unplanned and non-protocolled surgical lymphedema treatment. This patient was excluded while we were still under active patient enrollment, and thus another patient was included to replace the excluded patient. The 10 included patients were included and treated between December 2015 and May 2016 with follow-ups 1, 3, 6, and 12 months and 4 years after treatment. No patients were lost to follow-up, and the final follow-up was conducted in May 2020. There was no difference in median BMI at the start of the study (median, 30.95; IQR, 4.60; range, 22.32-35.91) or at the end of the study (median, 31.40; IQR, 12.99; range, 20.69-37.45). At the time of inclusion, patients had a median age of 55 (IQR, 10; range, 34-68) years and had unilateral BCRL with a median duration of 28.58 (IQR, 15.16; range, 8-36) months. For the ADRC treatment, a median of 280 (IQR, 35; range, 220-375) grams of adipose tissue was harvested from the patients, and of this, a median of 30 (IQR, 10; range, 20-40) grams of adipose tissue was used for the scar-releasing lipotransfer (Figure 2). The abdomen was used as the donor site in five patients, and the thighs were used in the remaining five patients. The ADRC isolation and characterization have been previously published.[3,4] In brief, cell isolation using a median of 252 (IQR, 41; range, 185-338) grams of adipose tissue yielded 2.20 (IQR, 0.59; range, 1.47-2.62) $\times 10^5$ cells per gram of fat, and a median of 5.32 (IQR, 1.68; range, 4.04-7.44) $\times 10^7$ cells were injected. The ADRCs were isolated after a median of 2 hours and 30 minutes (IQR, 0.17 minutes; range, 2 hours 0 minutes to 2 hours 55 minutes) and transferred immediately to the patient.

There was no significant decrease in median lymphedema volume before treatment (median, 299.50; IQR, 240.45; range, 134.1-705.0) and after 4 years of follow-up (median, 278.60; IQR, 361.21; range, 85.80-649.20; Figure 3A). There was no significant change in the volume of the lymphedematous or healthy arm before treatment or after 4 years of follow-up (Figure 3B,C; Table S1).

The patients reported a decrease in their lymphedema symptoms over time. The median score for arm heaviness improved from 5.50 (IQR, 3; range, 4-8) at baseline to 2.00 (IQR, 4.50; range, 0-7) after 12 months and 3.50 (IQR, 5.25; range, 0-7) after 4 years ($P < .05$; Figure 4A). Likewise, the median score for arm tension decreased from 5.00 (IQR, 1.5; range, 2-9) at baseline to 3.00 (IQR, 3.00; range, 0-7) after 12 months and 2.00 (IQR, 3.25; range, 0-8) after 4 years ($P < .05$; Figure 4B). Further improvements were noted in arm, shoulder, and hand function, as measured by the DASH questionnaire. The DASH score improved from 21.25 (IQR, 23.55; range, 5.00-41.67) at baseline to 13.33 (IQR, 28.55; range, 0-36.67) after 12 months and 9.17 (IQR, 18.33; range, 0.83-37.50) after 4 years ($P < .05$; Figure 4C). There were no significant changes in the LYMQOL quality of life, function, appearance, symptoms, or mood subscales from baseline and after 4 years of follow-up (Figure 4D-H).

Five of 10 patients had previously had cellulitis in their lymphedematous arm. After treatment, there were no cases of cellulitis during the first year. Overall, the yearly incidence of cellulitis reduced from a mean ± SD of 0.92 ± 1.34 per year before treatment to 0.46 ± 0.81 per year after treatment; however, the reduction did not quite reach statistical significance ($P = .065$; Figure 5).

Six of 10 patients downstaged their lymphedema treatment on their own initiative. One patient discontinued all use of compression sleeve and gauntlet. One patient reduced the frequency of her use of compression garments. One patient discontinued her use of a

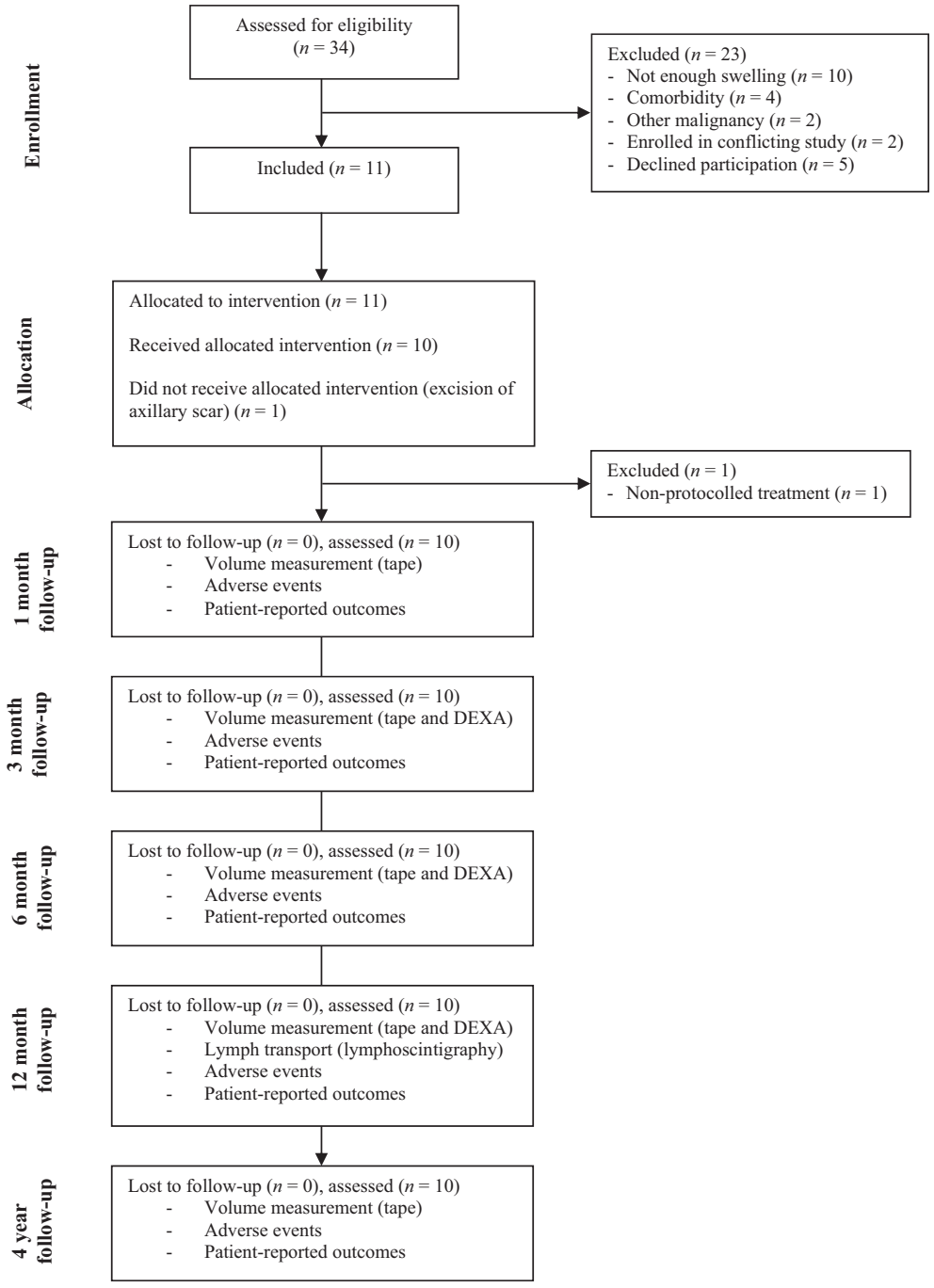

FIGURE 1 This figure shows the study enrollment, allocation, and protocolled measurements at each follow-up. Ten patients were included in this study. Volume measurements using multiple circumference measurements were done before treatment and after 1, 3, 6, 12, and 48 months of treatment. Volume measurements using DEXA scans were done before treatment and after 1, 3, 6, and 12 months of treatment. Adverse events were assessed after 1, 3, 6, 12, and 48 months of treatment. Lymph transport was assessed using lymphoscintigraphy before treatment and after 12 months of treatment. Lymphedema symptoms and patient-reported outcome measures were assessed before treatment and after 1, 3, 6, 12, and 48 months of treatment. DEXA, dual enegy x-ray absorptiometry

compression gauntlet. One patient discontinued the use of her pneumatic compression device. One patient downstaged her arm sleeve compression class (Table 1 has an overview). Three patients had no change in their lymphedema treatment, and one patient upstaged her use of compression sleeve.

Five patients felt their lymphedema had improved substantially after treatment, all of whom felt their lymphedema was less swollen. Four patients felt their improvements were of such significance that they would redo the treatment in the hope of additional improvements.

No serious adverse events were found. Minor short-term adverse events related to the liposuction and injections have been reported previously.[3] During the 4-year study period, patient no. 1 was diagnosed with early-stage contralateral breast cancer 10 months after treatment, and patient no. 10 was diagnosed with a distant recurrence of her primary breast cancer 42 months after treatment. No case of locoregional breast cancer occurred.

4 | DISCUSSION

This study is the first phase I study evaluating the safety and feasibility of ADRCs and lipotransfer for the treatment of BCRL. We did not find treatment to decrease actual lymphedema size; however, BCRL symptoms and upper extremity function improved.

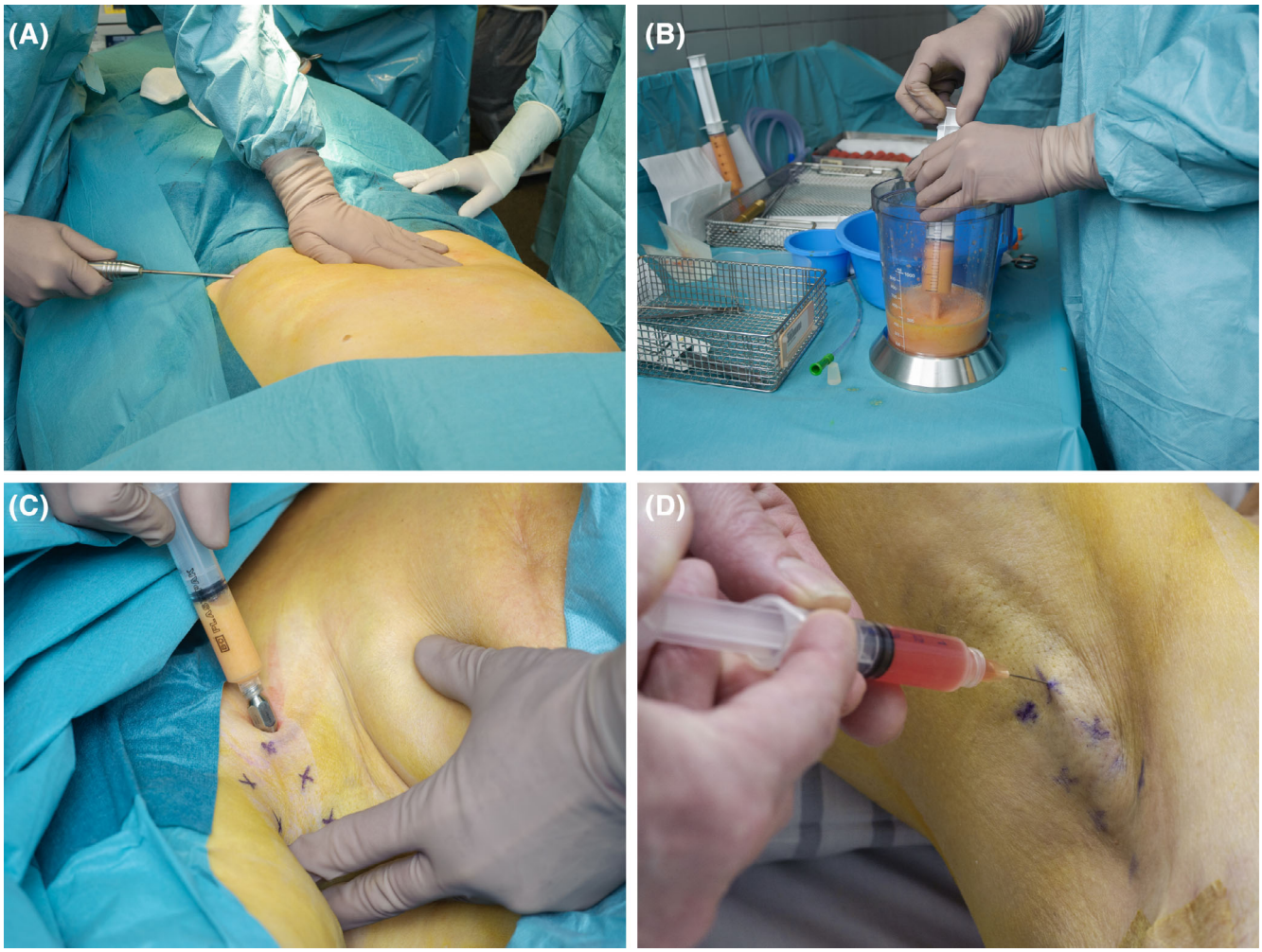

FIGURE 2 This figure shows the experimental lipotransfer and cell treatment for lymphedema. A) Liposuction using water-jet-assisted liposuction during general anesthesia. B) Aspiration of decanted adipose tissue. C) Axillary scar releasing lipotransfer injected in a fan-shaped pattern during general anesthesia. D) Subcutaneous injection of adipose-derived regenerative cells into the lipotransfered area in the patient ward

The main strengths of this study are its long follow-up time and complete reporting of baseline characteristics and subjective and objective assessments to avoid reporting bias. The study design naturally has a high risk of bias because of the lack of blinding in outcome assessments, a low number of patients, and the lack of a comparison group. Thus, the positive subjective improvements may be subject to detection bias, as patients may have been extra attentive to their treatment, and all were determined to attempt an experimental lymphedema treatment. We found improvements in BCRL symptoms and the DASH scale throughout the study; however, the LYMQOL subscales did not show consistent improvements. One reason for this could be that the LYMQOL is not validated in Danish. At the time of this study, there was no lymphedema-specific patient-reported outcome measure available in Danish, and we, therefore, used a translated version of the LYMQOL without validation. The primary goal of all lymphedema treatments is to improve the patient's condition. In this study, we chose volume reduction as the primary outcome because this is an objective measurement for improvements in the patient's lymphedema condition, given the inherent bias in our unblinded study design. However, lymphedema volume does not correlate well with patients' quality of life,[13] and it may be more appropriate for future studies to use a more patient-centered primary outcome measurement. We have previously published arm volume estimation using dual energy x-ray absorptiometry (DEXA) scans and lymphatic transport using lymphoscintigraphy up to 1 year after treatment. However, we did not perform DEXA or lymphoscintigraphy at the 4-year follow-up. We did not consider the DEXA scans and lymphoscintigraphy outcomes to add further value to the study, as they did not show any definite objective improvements during the first 12 months of treatment. Also, the DEXA scans showed an acceptable correlation with tape measurements for arm volume estimation.[4]

Overall, we did not find the adipose-derived treatment to decrease the volume of lymphedema, whereas other studies have found considerable reduction.[14-16] However, these studies all used bone marrow-derived regenerative cells (BDRCs); these cells also stem from the mesenchymal progenitor line and have regenerative properties comparable to adipose-derived cells.[17-19] Maldona et al

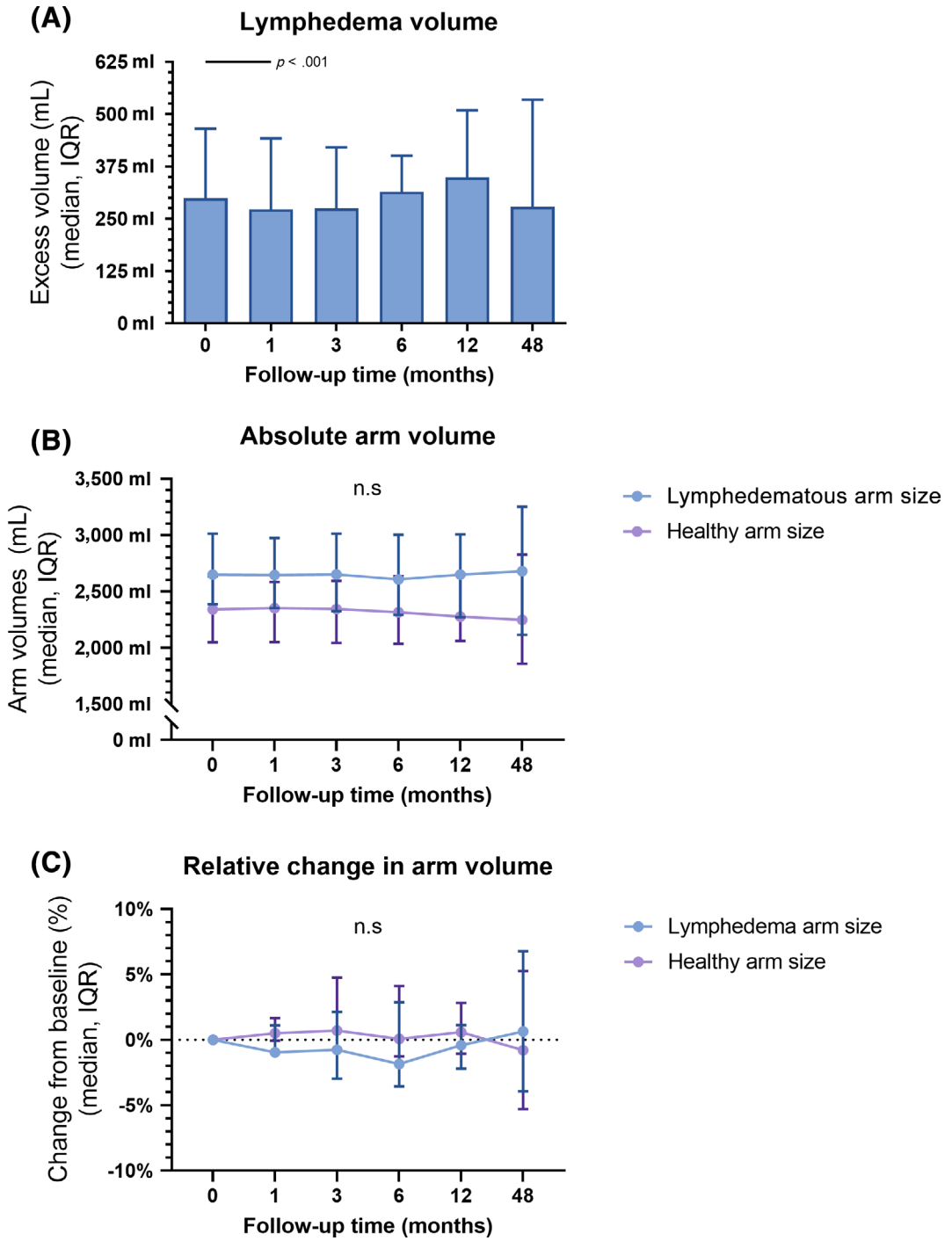

FIGURE 3 This figure shows the lymphedema and arm volumes. A) Lymphedema volume. Only a minor decrease in lymphedema volume after 1 month was noted; however, this effect did not persist during the 4-years of follow-up. The excess volume was defined as the difference between the lymphedema and the healthy arm. B) Absolute arm volumes of the lymphedema and healthy arm. No significant change in either volume was noted during the 4 years of follow-up. C) Relative change in arm volumes of the lymphedema and healthy arms. No significant change was noted during the 4 years of follow-up. N.s = not significant. IQR = interquartile range

showed that BDRC treatment without compression sleeve was as effective as compression sleeve alone in decreasing BCRL volume.[14] However, because of the open-label nature of their study, there was a high risk of performance bias. Of note, the BDRC group had longer lymphedema duration and latency compared with the compression group. Additionally, there seems to be an incongruity in volume estimation between the groups. The BDRC group had about 20% more lymphedema volume at baseline compared with the compression group, despite more patients being classified as having mild lymphedema (defined as <20% swelling) in the BDRC group (6/10)

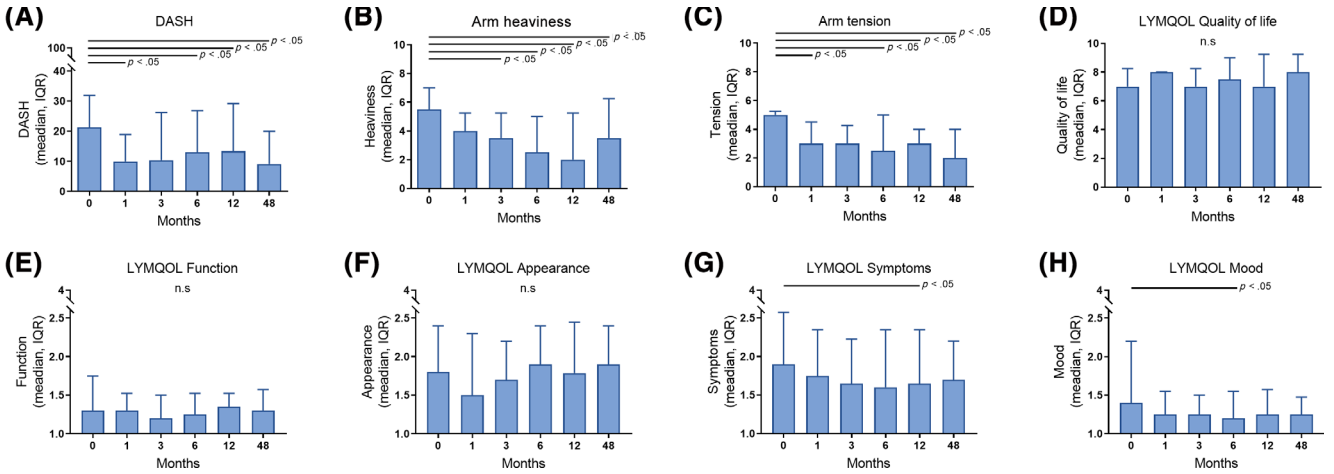

FIGURE 4 Lymphedema symptoms and patient-reported outcomes. Figure 4 legend: This figure shows the lymphedema symptoms and patient-reported outcomes. A) Improvement in self-reported disability of the arm, shoulder, and hand. B) Improvements in feelings of arm heaviness. C) Improvements in feelings of arm tension. D) No change in the lymphedema-related quality of life. E) No change in lymphedema-related function. F) No change in lymphedema-related appearance. G) No change in lymphedema-related symptoms. H) No change in lymphedema-related mood. P = p-value significance level. N.s = not significant

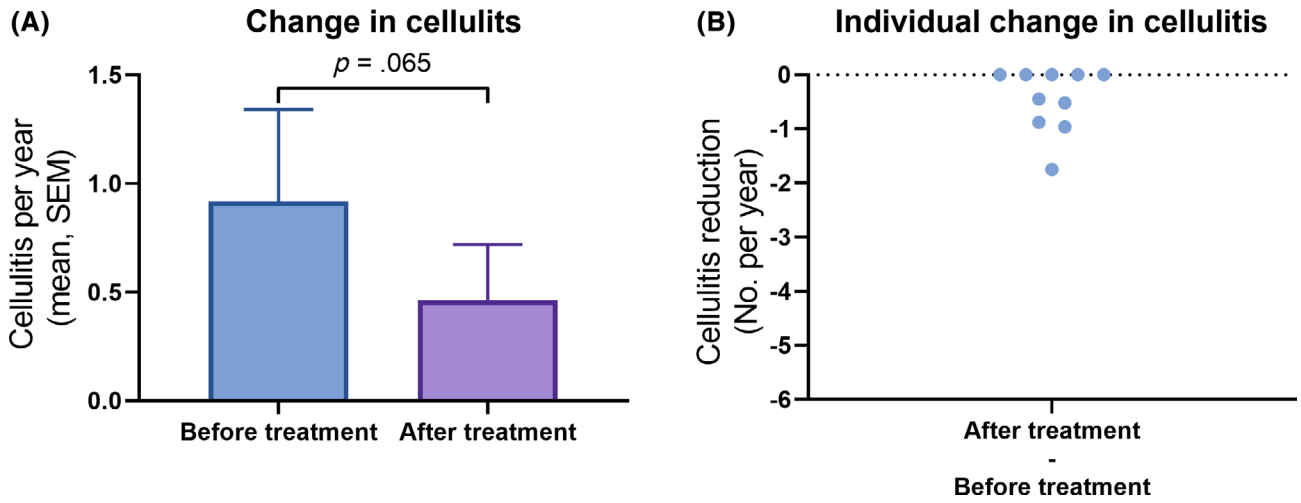

FIGURE 5 Lymphedema-associated cellulitis. Figure 5 legend: This figure shows the average number of lymphedema-associated cellulitis incidents before and after treatment. A) The average number of cellulitis incidents per year before and after treatment. The absolute reduction after treatment was not quite statistically significant. B) Overview of each patient's change in cellulitis incidents per year before and after treatment. Five patients had a prior history of cellulitis and had a small absolute reduction in the number of cellulitis after treatment. The remaining five patients had no prior history of cellulitis and had no cellulitis after treatment

compared with the compression group (1/10). The remaining patients had moderate lymphedema (defined as 20%-40% swelling), and the groups had equivalent BMIs at baseline. Therefore, these differences are unlikely to be due to differences in body type. Hou et al found that BDRCs increased the efficacy of complete decongestive therapy (CDT) in the treatment of BCRL.[15] However, their study also has unaddressed incongruity in volume estimation at baseline. The authors reported the swelling at baseline to be approximately 1100 mL, corresponding to a 29% arm size difference in both groups, which is an abnormally large volume for a moderate increase in percentage. Although the authors do not directly report the absolute volumes of the lymphedematous and unaffected arms, these values can be derived from the absolute and relative arm volume differences at baseline. Using their reported numbers, the healthy and lymphedematous arms measured approximately 4100 mL and 5200 mL at baseline. These volumes are far exceeding and almost double those of other CDT studies.[20-23] On the same note, the authors reported a volume reduction of CDT (with and without BDRCs) that far exceeds those of randomized controlled trials that evaluate CDT effectiveness.[20-23] The authors do not report their patients' BMI; however, it seems unlikely that such significant discrepancies in arm volumes from the literature would be due to morbid obesity, as the BMI in other studies is around 30 to 35.[20-23]

TABLE 1 Patient characteristics

Patient identifier	Time until LE, years	LE duration, years	LE in dominant arm	Pitting	ISL stage	LE management before	LE management after
1	1.32	1.39	Yes	Yes	2	Sleeve (≤3 days/week) Gauntlet (≤3 days/week) Pneumatic device (daily)	None
3	0.55	2.40	Yes	(slight)	1	Sleeve (daily)	Sleeve (daily, CCL1)
4	0.27	3.01	Yes	Yes	1	Sleeve (daily)	Sleeve (daily)
5	0.86	2.88	No	No	2	Sleeve (<1 day/week)	Sleeve (daily)
6	0.91	2.24	No	Yes	2	Sleeve (daily) Pneumatic device (daily)	Sleeve (daily)
7	3.21	0.80	No	(slight)	2	Sleeve (daily) Gauntlet (daily)	Sleeve (daily)
8	0.81	2.73	No	Yes	2	Sleeve (daily) Gauntlet (daily) Night sleeve (≤3 days/week)	Sleeve (daily) Gauntlet (daily) Night sleeve (≤3 days/week)
9	0.35	1.73	Yes	(slight)	2	Sleeve (daily) Gauntlet (daily) Pneumatic device (<1 day/week) Night sleeve (daily)	Sleeve (daily) Gauntlet (daily) Pneumatic device (<1 day/week) Night sleeve (daily)
10	0.75	3.12	Yes	(slight)	2	Sleeve (daily) Gauntlet (≤3 days/week)	Sleeve (≤3 days/week)
11	2.54	4.51	No	(slight)	2	Sleeve (daily) Gauntlet (daily) Night sleeve (daily)	Night sleeve (daily)

Note: This table shows an overview of the included patients and change in type and use of lymphedema treatment before and 4 years after treatment. The compression class for all garments was compression class 2 unless otherwise stated.
Abbreviations: CCL, compression class; ISL, International Society of Lymphology; LE, lymphedema.

In a similar recent study, Ismail et al discovered that BDRCs also increased the efficacy of CDT in primary lower limb lymphedema.[16] However, this study too may have been subject to performance bias, namely, a systematic difference in exposure to compression therapy between the BDRC and CDT groups. Compression therapy is a known confounder for volume reduction in lymphedema, as significant volume reduction can be credited to this treatment alone.[24,25] Unfortunately, little detail about the patients' use of compression therapy before and after treatment is reported. The authors note that multilayered bandaging was applied for 2 weeks and then released for 2 weeks alternatively for the 6-month study period; however, multilayered bandaging is usually performed just for a few weeks.[26,27] It is not clear why multilayered bandaging was applied for so long in this study and, importantly, when in this rotation the lymphedema measurements were undertaken. Skin indention from multilayered bandaging is evident in several patient photos from the BMC group after, but not before treatment. This suggests that at least some of the size reduction can be attributed to the CDT treatment. Interestingly, however, the control group showed no improvements after CDT, which further suggests a systematic difference in compression therapy during the study or some unaccounted baseline differences.

In our study, the positive subjective improvements did not translate into a decreased lymphedema volume. One possible explanation for this is that most patients were still using elastic compression sleeves at baseline and all follow-ups, which could make decreases in arm size undetectable because of the elastic nature of compression garments. Also, most patients were in a latent phase of their lymphedema and had limited pitting, suggesting that the majority of the swelling was manifest with very little drainable fluid.[6] It is also possible that the subjective improvements in arm function were a result of the mechanical scar-releasing lipotransfer or merely the patient's treatment expectations. All of our patients had axillary scarring after their previous breast cancer treatment with axillary lymph node dissection and radiation for most. Lipotransfer has consistently been shown to alleviate fibrotic and adhesive scars after surgery and radiation.[28] Also, axillary reaugmentation and lipotransfer have been shown to successfully alleviate lymphedema after breast cancer treatment,[29,30] perhaps by decompressing lymphatic and venous pressure and reconnecting distal and proximal lymphatic drainage. In our study, the subjective improvements were stable throughout the 4-year study period after a one-time intervention. Usually, more than one lipotransfer session is needed to achieve satisfactory scar release.[28] Therefore, it would be interesting in the future to assess whether additional benefits can be achieved with repeated interventions. ADRCs downregulate inflammation and oxidative stress[31] and have been shown to increase lymphangiogenesis in irradiated human

dermal endothelial cells.[32] These immunoregulative properties of ADRCs may explain the unexpected improvements in cellulitis achieved in all five patients with existing cellulitis at the time of intervention. Additionally, no patients without a previous history of cellulitis developed cellulitis during the 4-year follow-up. In general, lymphedema worsens during noncompliance with compression therapy.[33] The fact that lymphedema was stable, even when some patients downstaged their compression treatment, at least hints at a possible benefit of the treatment. During the study period, one patient was diagnosed with new breast cancer, and one patient developed a distant recurrence of her primary breast cancer. These events are unfortunate but not unexpected, given known risks for secondary contralateral breast cancer and the patients' baseline tumor burden.[34,35] Treatment safety is paramount, and future adequate-sized randomized, controlled trials with proper blinding are needed to answer these challenging questions.

5 | CONCLUSION

The evidence from this long-term, open-label, phase I study implies that ADRCs and lipotransfer are safe and feasible in the setting of previous breast cancer and can potentially alleviate BCRL and cellulitis. After treatment, patients reported improvements in arm, shoulder, and hand function, and 6 of 10 patients had decreased their use of conservative lymphedema treatment. There was no measurable change in lymphedema volume during the study; however, 5 of 10 patients felt the volume had subsided. Four of 10 patients felt their improvements were of such significance that they would undergo the treatment again in the hope of additional improvements. These promising results will need to be tested in a sufficient randomized, controlled, and blinded study to rule out a placebo effect before a routine clinical transition.

CONFLICT OF INTEREST

C.H.J. and D.C.A. are inventor or patent holder with Odense University Hospital/University of Southern Denmark and have ownership interest with Blue Cell Therapeutics. The other authors indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

M.G.J.: conception/design, provision of study material or patient, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; N.M.T.: conception/design, provision of study material or patients, collection and/or assembly of data, data analysis and interpretation, final approval of manuscript; C.H.J.: conception/design, provision of study material or patients, collection and/or assembly of data, final approval of manuscript; D.C.A.: collection and/or assembly of data, administrative support, final approval of manuscript; S.P.S.: conception/design, financial support, provision of study material or patients, final approval of manuscript; J.A.S.: conception/design, financial support, provision of study material or patients, administrative support, final approval of manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Toyserkani NM, Jørgensen MG, Haugaard K, Sørensen JA. Seroma indicates increased risk of lymphedema following breast cancer treatment: a retrospective cohort study. *Breast*. 2017;32:102-104.
2. Toyserkani NM, Christensen ML, Sheikh SP, Sørensen JA. Stem cells show promising results for lymphoedema treatment–a literature review. *J Plast Surg Hand Surg*. 2015;49:65-71.
3. Toyserkani NM, Jensen CH, Andersen DC, Sheikh SP, Sørensen JA. Treatment of breast cancer-related lymphedema with adipose-derived regenerative cells and fat grafts: a feasibility and safety study. *stem cells translational med*. 2017;6:1666-1672.
4. Toyserkani N, Jensen C, Tabatabaeifar S, et al. Adipose-derived regenerative cells and fat grafting for treating breast cancer-related lymphedema: lymphoscintigraphic evaluation with 1 year of follow-up. *J Plast Reconstr Aesthet Surg*. 2019;72:71-77.
5. Toyserkani NM, Jørgensen MG, Tabatabaeifar S, Jensen CH, Sheikh SP, Sørensen JA. Concise review: a safety assessment of adipose-derived cell therapy in clinical trials: a systematic review of reported adverse events. *stem cells translational med*. 2017;6:1786-1794.
6. International Society of Lymphology. The diagnosis and treatment of peripheral lymphedema: 2013 Consensus Document of the International Society of Lymphology. *Lymphology*. 2013;46:1-11.
7. Dregalla RC, Lyons NF, Reischling PD, Centeno CJ. Amide-type local anesthetics and human mesenchymal stem cells: clinical implications for stem cell therapy. *stem cells translational med*. 2014;3:365-374.
8. Bourin P, Bunnell BA, Casteilla L, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy*. 2013;15:641-648.
9. Brorson H, Höijer P. Standardised measurements used to order compression garments can be used to calculate arm volumes to evaluate lymphoedema treatment. *J Plast Surg Hand Surg*. 2012;46:410-415.
10. Tambour M, Tange B, Christensen R, Gram B. Effect of physical therapy on breast cancer related lymphedema: protocol for a multicenter, randomized, single-blind, equivalence trial. *BMC Cancer*. 2014;14:239.
11. Keeley V, Crooks S, Locke J, et al. A quality of life measure for limb lymphoedema (LYMQOL). *J Lymphoedema*. 2010;5:27-37.
12. Schønnemann JO, Larsen K, Hansen TB, Søballe K. Reliability and validity of the Danish version of the disabilities of arm, shoulder, and hand questionnaire in patients with fractured wrists. *J Plast Surg Hand Surg*. 2011;45:35-39.
13. Lee TS, Morris CM, Czerniec SA, Mangion AJ. Does lymphedema severity affect quality of life? Simple question. Challenging answers. *Lymphat Res Biol*. 2018;16:85-91.

14. Maldonado GEM, Pérez CAA, Covarrubias EEA, et al. Autologous stem cells for the treatment of post-mastectomy lymphedema: a pilot study. *Cytotherapy*. 2011;13:1249-1255.
15. Hou C, Wu X, Jin X. Autologous bone marrow stromal cells transplantation for the treatment of secondary arm lymphedema: a prospective controlled study in patients with breast cancer related lymphedema. *Jpn J Clin Oncol*. 2008;38:670-674.
16. Ismail AM, Abdou SM, Abdelnaby AY, Hamdy MA, el Saka AA, Gawaly A. Stem cell therapy using bone marrow-derived mononuclear cells in treatment of lower limb lymphedema: a randomized controlled clinical trial. *Lymphat Res Biol*. 2018;16:270-277.
17. Zhou W, Lin J, Zhao K, et al. Single-cell profiles and clinically useful properties of human mesenchymal stem cells of adipose and bone marrow origin. *Am J Sports Med*. 2019;47:1722-1733.
18. Waldner M, Zhang W, James IB, et al. Characteristics and immunomodulating functions of adipose-derived and bone marrow-derived mesenchymal stem cells across defined human leukocyte antigen barriers. *Front Immunol*. 2018;9:1642.
19. Ibrahim A, Rodriguez-Florez N, Gardner OFW, et al. Three-dimensional environment and vascularization induce osteogenic maturation of human adipose-derived stem cells comparable to that of bone-derived progenitors. *stem cells translational med*. 2020;9:1651-1666.
20. Uzkeser H, Karatay S, Erdemci B, Koc M, Senel K. Efficacy of manual lymphatic drainage and intermittent pneumatic compression pump use in the treatment of lymphedema after mastectomy: a randomized controlled trial. *Breast Cancer*. 2015;22:300-307.
21. Gradalski T, Ochalek K, Kurpiewska J. Complex decongestive lymphatic therapy with or without Vodder II manual lymph drainage in more severe chronic postmastectomy upper limb lymphedema: a randomized noninferiority prospective study. *J Pain Symptom Manage*. 2015;50:750-757.
22. Tambour M, Holt M, Speyer A, Christensen R, Gram B. Manual lymphatic drainage adds no further volume reduction to complete decongestive therapy on breast cancer-related lymphoedema: a multicentre, randomised, single-blind trial. *Br J Cancer*. 2018;119:1215-1222.
23. McNeely ML, Magee DJ, Lees AW, et al. The addition of manual lymph drainage to compression therapy for breast cancer related lymphedema: a randomized controlled trial. *Breast Cancer Res Treat*. 2004;86:95-106.
24. Maegawa J, Hosono M, Tomoeda H, Tosaki A, Kobayashi S, Iwai T. Net effect of lymphaticovenous anastomosis on volume reduction of peripheral lymphoedema after complex decongestive physiotherapy. *Eur J Vasc Endovasc Surg*. 2012;43:602-608.
25. Vignes S, Brorson H. Comment on "clinical and psychosocial outcomes of vascularized lymph node transfer for the treatment of upper extremity lymphedema after breast cancer therapy". *Ann Surg Oncol*. 2017;24:559-560.
26. Oh SH, Ryu SH, Jeong HJ, Lee JH, Sim YJ. Effects of different bandaging methods for treating patients with breast cancer-related lymphedema. *Ann Rehabil Med*. 2019;43:677-685.
27. Torres-Lacomba M, Navarro-Brazález B, Prieto-Gómez V, Ferrandez JC, Bouchet JY, Romay-Barrero H. Effectiveness of four types of bandages and kinesio-tape for treating breast-cancer-related lymphoedema: a randomized, single-blind, clinical trial. *Clin Rehabil*. 2020;34:1230-1241.
28. Krastev TK, Schop SJ, Hommes J, Piatkowski A, van der Hulst RRWJ. Autologous fat transfer to treat fibrosis and scar-related conditions: a systematic review and meta-analysis. *J Plast Reconstr Aesthet Surg*. 2020;73:2033-2048.
29. Marx M, Geßner M, Florek A, et al. Re-augmentation of the axilla using a turn-over flap – a new approach for the surgical treatment of secondary lymphedema after breast cancer. *Geburtshilfe Frauenheilkd*. 2020;80:526-531.
30. Maruccia M, Elia R, Ciudad P, et al. Postmastectomy upper limb lymphedema: combined vascularized lymph node transfer and scar release with fat graft expedites surgical and patients' related outcomes. A retrospective comparative study. *J Plast Reconstr Aesthet Surg*. 2019;72:892-901.
31. Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. *Circ Res*. 2008;103:1204-1219.
32. Saijo H, Suzuki K, Yoshimoto H, Imamura Y, Yamashita S, Tanaka K. Paracrine effects of adipose-derived stem cells promote lymphangiogenesis in irradiated lymphatic endothelial cells. *Plast Reconstr Surg*. 2019;143:1189e-1200e.
33. Ochalek K, Gradalski T, Szygula Z. Five-year assessment of maintenance combined physical therapy in postmastectomy lymphedema. *Lymphat Res Biol*. 2015;13:54-58.
34. Holleczek B, Stegmaier C, Radosa JC, Solomayer EF, Brenner H. Risk of locoregional recurrence and distant metastases of patients with invasive breast cancer up to ten years after diagnosis - results from a registry-based study from GGermany. *BMC Cancer*. 2019;19:520.
35. Rusner C, Wolf K, Bandemer-Greulich U, et al. Risk of contralateral second primary breast cancer according to hormone receptor status in Germany. *Breast Cancer Res*. 2014;16:452.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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