European Review for Medical and Pharmacological Sciences 2018; 22: 4768-4777

Oncological safety of stromal vascular fraction enriched fat grafting in two-stage breast reconstruction after nipple sparing mastectomy: long-term results of a prospective study

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Abstract. – OBJECTIVE: Autologous fat transfer (AFT) is commonly used to treat implant palpability and prevent fibrosis and thinning in mastectomy skin flaps. A major limit to this procedure is volume retention over time, leading to the introduction of fat enrichment with stromal vascular fraction (SVF+AFT). Oncological concerns have been raised over the injection of an increased concentration of progenitors cells (ASCs) in the SVF. The aim of the study is to evaluate the long-term cancer recurrence risk of SVF+AFT cases compared to AFT, in patients undergoing Nipple Sparing Mastectomy (NSM).

PATIENTS AND METHODS: A prospective study was designed to compare three groups of patients undergoing NSM followed by SVF+AFT, AFT or none (control group), after a two-stage breast reconstruction. Patients were strictly followed-up for at least 5-years from the second stage reconstructive procedure. Loco-regional and systemic recurrence rate were evaluated over time as the primary outcome. Logistic regression was used to investigate which factors were associated with recurrence events and independent variables of interest were: surgical technique, age above 50 years old, lympho-vascular invasion, oncological stage, adjuvant or neoadjuvant chemotherapy, adjuvant radiotherapy and adjuvant hormone therapy.

RESULTS: 41 women were included in G1 (SVF+AFT), 64 in G2 (AFT), and 64 in G3 (control group). Loco-regional recurrence rate was 2.4% for G1, 4.7% for G2, and 1.6% for G3. Systemic recurrence was 7.3%, 3.1%, and 3.1%, respectively. Among the variables included, there were no significant risk factors influencing a recurrence event, either loco-regional or systemic. In particular, SVF+AFT (G1) did not increase the oncological recurrence.

CONCLUSIONS: Our data suggest that both centrifuged and SVF-enhanced fat transfer have a similar safety level in comparison to patients who did not undergo fat grafting in breast reconstruction after NSM.

Key Words:
Stromal vascular fraction, SVF, Fat grafting, Adipose derived stem cells, Oncological safety, Enriched fat grafting, Breast cancer.

Abbreviations

AT: Adipose Tissue, ADSC: Adipo-Derived Stem Cells, ADRC: Adipo-Derived Regenerative Cells, SVF: Stromal-Vascular Fraction, AFT: Autologous Fat Transfer, NSM: Nipple Sparing Mastectomy, TE: Tissue Expander, CIN: Cervical Intraepithelial Neoplasia, VIN: Vulvar Epithelial Neoplasia, CD: Cluster of Differentation, αSMA: Alpha-Smooth Muscle Actin, CT: Connective Tissue, IF: Increment Fold.

Introduction

Nipple sparing mastectomy (NSM) has gained a wide acceptance in the breast cancer scenario, due to its safety in terms of surgical

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and oncological outcomes[1-8]. In this regard, the amazing psychological advantage of preserving the nipple has led Veronesi to define this procedure a "conservative mastectomy". This apparently paradoxical concept clearly opens doors to new horizons in breast reconstruction[9,10].

Skin flaps after NSM are often quite thin and could result in palpable and visible implants, skin flap necrosis, or other disappointing cosmetic outcomes. Since 1990's, Autologous Fat Transfer (AFT) has been adopted as a common surgical procedure for soft tissue augmentation, to improve the subcutaneous layer softness and give a natural appearance and breast contour to patients who underwent mastectomy. AFT represents an ideal tool to correct local defects and improve the coverage and skin quality of the mastectomy flaps. There is an increasing body of both observational and reported evidence[1,11,12] for cutaneous trophic changes following subcutaneous implantation of adipo-derived elements, yet there is a lack of evidence of long-term fat volume retention and graft survival over time. In recent years, investigation has been focusing attention on enriching the autologous adipose tissue graft with stem/progenitor cells isolated from the stromal vascular fraction (SVF) of AT, in order to improve its characteristics and long term persistence. Indeed, numerous preclinical studies[12-16], both on animal models and humans, as well as an emerging body of clinical data, suggest that adipose stem/progenitor cells (ASC) from the SVF, improve fat volume retention over time.

It has been suggested that the AT serves as a scaffold upon which more concentrated ASC can organize and differentiate, thus promoting the secretion of soluble factors that enhance angiogenesis, decrease apoptosis, and/or modulate the immune response[17,18]. However, the presence of ASC within AFT, either with enrichment or in the standard fashion, albeit in low concentration, has raised another important issue, namely the requirement for oncological evaluation of a possible recurrence risk[19-22]. Even though in the recent past such a risk has been reported for the AFT procedure in cases of ductal carcinoma *in situ* (DCIS)[23], in 2016, Kronowitz et al[24] showed no increase in rates of loco-regional recurrence, systemic recurrence or second breast cancer, resulting in supporting the oncologic safety of fat grafting in breast reconstruction. The only subgroup examined in which lipofilling was associated with an increased risk of loco-regional recurrence was the subgroup treated with hormonal therapy, although the recurrence rates were low.

Delivering even more ASC to the tumor resection site may increase this fear of recurrence. The safety of SVF-enriched grafting for breast reconstruction has been largely debated but still there is no wide consensus as far. The aim of the study is to assess long-term oncological safety in patients undergoing SVF + AFT treatment compared to the common procedure of autologous fat transfer (AFT), following NSM and breast reconstruction. A multi-arm clinical study was carried out to evaluate the incidence of local and systemic recurrences after NSM reconstruction among patients undergoing SVF-AFT and AFT. A non-adipose tissue reconstruction was introduced as negative control group.

Patients and Methods

Patients

In 2007 we designed a prospective, multi-arm, single-center cohort study to compare and evaluate the effective oncological safety of SVF-enriched adipose tissue grafting. Patients diagnosed for breast cancer, who underwent NSM with breast reconstruction at our Institutions, were divided in three groups. Although we routinely perform direct-to-implant reconstruction, in this study we selected only patients eligible for two-stage breast reconstruction. The design of the study consisted in a first group of patients submitted to SVF-enriched adipose tissue grafting, or SVF-AFT (G1), a second group of patients submitted to the traditional Coleman's lipofilling technique, or AFT (G2), and a third control group of patients that did not received any fat grafting procedure (G3). Inclusion criteria of the study were: age between 18-75 years old, history of NSM for a histologically proven Tis-T2N0-N2M0 breast adenocarcinoma in the previous 24 months, breast reconstruction performed by means of a Tissue Expander (TE) temporary breast prosthesis, active oncological follow-up (according to Associazione Italiana Oncologia Medica, AIOM, follow-up schedule guidelines, http://www.aiom.it) with no documented recurrences nor systemic disease at the time of enrollment, and signed informed consent form. Exclusion criteria were: medical history

of other malignant diseases (except for Cervical Intraepithelial Neoplasia – CIN – and Vulvar Intraepithelial Neoplasia – VIN –), including a previous breast cancer and NSM procedures, severe comorbidities (heart failure, hepatic and renal failure, collagen systemic diseases, psychiatric disorders), and no active and scheduled follow-up. Whenever fat grafting was considered useful for the reconstruction, the patient was considered eligible for the study, if fitting all the inclusion and exclusion criteria. Control Group (G3) was collected from patients who did not require fat grafting upon enrollment. G3 Patients did not undergo any fat graft procedure. Experimental Groups (G1 and G2) were subject to AFT+SVF (G1) or AFT alone (G2) at the time of second stage breast reconstruction. Subjects were not randomized and different procedures were performed dependent upon the operating reconstructive surgeon (CC for G1, DC, for G2), after a thorough evaluation with the patient, and a signed informed consent.

A 5-years minimum follow-up, beginning from the second stage procedure, was planned including a standard AIOM schedule plus an ultrasound imaging of both breasts, once a year. MRI was limited to suspicious cases. Follow-up was calculated considering last visit update, according to each patient's records. The study followed the ethical standards of human experimentation, according to the Declaration of Helsinki.

Surgical Procedures

Adipose Tissue Harvest

In both groups G1 and G2, patients were submitted to a fat graft procedure at the time of second stage reconstruction, during the expander/implant exchange procedure[25]. All procedures were performed under general anesthesia. Liposaspiration was performed in either the abdomen, thighs, flanks, inner knees or gluteal regions, depending on patient's body habitus and preferences. An antibiotic prophylaxis was administered in all cases 30 minutes before surgical incisions.

Preparation of SVF-Enriched Fat Graft (Group 1, SVF+AFT)

The technique was conducted as previously described in the RESTORE-2 trial[25] Aspirated AT was divided in two parts. Part one was added to the Celution® system (Cytori Therapeutics; San Diego, CA, USA). The Adipose-Derived Regenerative Cells (ADRCs) were released from their bound matrix with the addition of a proteolytic enzyme reagent (Celase®, Cytori Therapeutics; San Diego, CA), washed to remove residual enzyme, and then concentrated within the closed automated system in the operating room. The second part of lipoaspirate was purified with gravity sedimentation/flotation. Part one (concentrated ADRCs – approximately 5 mL), was added and mixed to part two to create the ADRC-enriched fat graft. This enriched fat graft was then transferred in a sterile way to the surgical field using 60 mL Toomey syringes.

Preparation of Standard Fat Graft (Group 2, AFT Alone)

Autologous lipoaspirate was centrifuged and processed according to the standard Coleman's technique[25].

Delivery of fat graft (Either Enriched or Aalone)

After tissue expander removal and definitive implant positioning via an inframammary crease access, a mastectomy flap dissection was performed using a blunt cannula in a fan-shaped direction to include all over the breast mound, represented by the new implant. Dissection was carried out from the surgical incision, in the subcutaneous space between skin and implant capsula along with pectoralis muscle fibers. AFT was performed in this pre-tunneled plane using the Celbrush® (Cytori Therapeutics; San Diego, CA, USA) for G1 patients and standard cannulas for G2 patients. Antibiotic therapy was continued per os from the first post-operative day until drains were removed.

Control Group

Patients belonging to Group 3 underwent the same pre, intra and post-operative treatment except for the fat grafting procedure, which was not performed.

Follow-up

After discharge, patients were followed-up once a week for a month, then once a month for the following three months, and thereafter according to the oncological follow-up schedule mentioned above. Senior author (RD) revised outcomes and results.

Statistical Analysis

Standard descriptive statistics were used to summarize data. Comparison of clinical characteristics between the three groups of patients was performed by Kruskal Wallis rank test for continuous variables and Pearson's x^2-test (or Fisher's exact test when appropriate) for categorical variables. Logistic regression was used to investigate which factors were associated with recurrence events, either loco-regional or systemic. Independent variables of interest were: surgical technique, age above 50 years old, lympho-vascular invasion, oncological stage, adjuvant or neoadjuvant chemotherapy, adjuvant radiotherapy, and adjuvant hormone therapy. A *p*-value less than 0.05 was considered statistically significant. All analyses were performed using STATA version 13 (StataCorp. 2013. Stata Statistical Software: Release 13. StataCorp LP, College Station, TX, USA).

Results

Between January 2008 and April 2011, 54 patients were enrolled in G1 Group, 57 in G2, and 72 in G3. Four patients were lost to follow-up in G1, two patients were lost in G2, and four were lost in G3. Nine patients enrolled in G1 declined to undergo the SVF-enriched fat grafting, choosing the standard fat grafting procedure and were therefore included in G2. Four patients were subsequently submitted to a fat graft procedure during follow-up in G3 and hence excluded from the study. In summary, patients included in the present analysis were 41 women in G1, 64 women in G2, and 64 women in G3. A comparison of baseline patients characteristics and oncological data is reported in Table I. No statistically significant difference was found among the groups. Median intervals from first stage procedure were 10, 9, and 12

Table I. Baseline patient's characteristics and oncological data.

	G1	G2	G3
Age, mean (range)	48.8 (34-61)	50.3 (33-69)	47.7 (33-60)
T			
Tis	5 (7.3%)	9 (14.1%)	6 (9.4%)
T1	16 (24.4%)	38 (59.4%)	26 (40.6%)
T2	20 (68.3%)	17 (26.5%)	32 (50%)
DCIS component	12 (29.3%)	30 (46.9%)	19 (29.7%)
N			
N0	20 (48.8%)	32 (50.0%)	33 (51.6%)
N1	12 (29.3%)	26 (40.6%)	27 (42.2%)
N2	9 (21.9%)	6 (9.4%)	4 (6.2%)
Grading			
Not reported	3 (7.3%)	9 (14.1%)	5 (7.8%)
1	2 (4.9%)	11 (17.2%)	9 (14.1%)
2	20 (48.8%)	18 (21.1%)	23 (35.9%)
3	16 (39.0%)	26 (40.6%)	27 (42.2%)
Sentinel node biopsy	38 (92.7%)	52 (81.3%)	56 (87.5%)
Axillary lymph nodes dissection	19 (46.3%)	32 (50.0%)	30 (46.9%)
Lympho-vascular invasion	19 (46.3%)	23 (35.9%)	25 (39.1%)
Adjuvant or neoadjuvant chemotherapy	28 (68.3%)	37 (57.8%)	35 (54.7%)
Adjuvant hormone therapy	30 (73.2%)	43 (67.2%)	44 (68.8%)
Adjuvant radiation therapy	17 (41.5%)	9 (14.1%)	4 (6.3%)
ER			
Not reported	3 (7.3%)	9 (14.1%)	5 (7.8%)
Negative	9 (22.0%)	12 (18.7%)	15 (23.4%)
Positive	29 (70.7%)	43 (67.2%)	44 (68.8%)
PGR			
Not reported	3 (7.3%)	7 (10.9%)	5 (7.8%)
Negative	10 (24.4%)	16 (25.0%)	15 (23.4%)
Positive	28 (68.3%)	41 (64.1%)	44 (68.8%)

Table II. Details of follow-up and recurrences.

	G1	G2	G3
Interval between first and second stage, median (range)	10 (1-22)	9 (5-14)	12 (8-15)
Follow-up after second procedure, median (range)	84 (60-96)	75 (60-96)	72 (60-96)
Loco-regional recurrences	1 (2.4%)	3 (4.7%)	1 (1.6%)
Systemic recurrences	3 (7.3%)	2 (3.1%)	2 (3.1%)

months for G1, G2, and G3 respectively. Median follow-up after the second procedure were 84 months (range 60-96), 75 months (range 60-96), and 72 months (range 60-96), respectively. The G1 Group demonstrated one local axillary lymph node recurrence (2.4%) (Table II). Three systemic recurrences were also recorded (7.3%) in this group: two bone metastases and one case of pulmonary plus liver metastases. Disease free survival (DFS) was 19, 22, and 25 months after the last reconstructive stage and 37, 34, and 38 months after NSM. The G2 Group presented three local recurrences (4.7%) and two systemic recurrence events (3.1%). One of these patients presented both a local and systemic recurrence. A nodule at the level of the mastectomy flap was documented as a local recurrence 28 months after NSM and 15 months from the second stage and lipofilling procedure; two loco-regional axillary lymph nodes recurrence were registered 24 and 27 months from fat graft and 32 and 40 months from NSM, respectively. Systemic recurrences were one pulmonary and one brain metastasis, occurring 14 and 24 months from the second stage and 22 and 32 from NSM, respectively. Finally, the G3 Control group showed the presence of one local recurrence (1.6%) located in the nipple and two cases of systemic recurrences (3.1%): one bone and one bone plus liver metastasis. DFSs were 11, 13, 9 months from the second stage and 23, 34, 26 months from NSM respectively.

Regression analysis showed that among the oncological variables included, there were no significant risk factors, which would lead to a recurrence event, either loco-regional or sys-

Table III. Analysis of the risk of any recurrence (either local or systemic) related to several variables, including the three different surgical approaches.

	Events	OR (95% CI), *p*-value	Adjusted OR (95% CI), *p*-value
Group			
Group 1	4/41 (9.8%)	2.20 (0.47, 10.4) 0.320	1.92 (0.36, 10.31) 0.447
Group 2	4/64 (6.3%)	1.36 (0.29, 6.32) 0.698	1.26 (0.25, 6.42) 0.778
Group 3	3/64 (4.7%)	Ref.	Ref.
Age			
≤ 50	4/106 (3.8%)	Ref.	Ref.
> 50	7/63 (11.1%)	3.19 (0.89, 11.36) 0.074	3.40 (0.87, 13.25) 0.078
Stage			
0-I	4/83 (4.8%)	Ref.	Ref.
II-III	7/86 (8.1%)	1.75 (0.49, 6.22) 0.387	1.20 (0.29, 4.95) 0.797
Lympho-vascular invasion			
No	5/102 (4.9%)	Ref.	Ref.
Yes	6/67 (9.0%)	1.91 (0.56, 6.52) 0.303	2.16 (0.59, 7.87) 0.224
Adjuvant or neoadjuvant chemotherapy			
No	4/69 (5.8%)	Ref.	Ref.
Yes	7/100 (7.0%)	1.22 (0.34, 4.35) 0.756	1.30 (0.35, 4.90) 0.695
Adjuvant hormone therapy			
No	4/51 (7.7%)	Ref.	Ref.
Yes	7/117 (6.0%)	0.76 (0.21, 2.73) 0.678	0.85 (0.22, 3.37) 0.818
Adjuvant radiation therapy			
No	8/139 (5.8%)	Ref.	Ref.
Yes	3/30 (10.0%)	1.82 (0.45, 7.31) 0.399	1.14 (0.24, 5.50) 0.873

OR: Odds Ratio; CI: Confidence Interval; Ref.: Reference category.

temic. In particular, the adopted surgical technique does not represent a significant risk factor for recurrences (Table III). SVF+AFT (G1) did not increase the oncological recurrence, thus it seems to be safe from an oncological point of view.

Discussion

Much attention has been given to the use of fat grafting as a source of cells with powerful biological effect in both cosmetic and reconstructive surgery. However, the putative powerful biological effect of constitutive or additive adipo-derived cells may not be sufficient to secure total engraftment and its consequent volume restoration, it might potentiate reparative, angiogenic or immunosuppressive mechanisms to increase oncological loco-regional recurrence. Indeed, fat grafting techniques continue to improve breast aesthetic outcomes in oncologically safe population, yet its adoption in postoncological breast reconstruction remains limited by two main concerns: unreliable fat volume retention over time and the increased risk of local recurrence in oncological patients. Stromal Vascular Fraction Enriched Fat Grafting has been introduced providing well-documented improvements in volume retention[12-14,16]. However, minimal safety evidence can be reviewed in current literature. Volume retention rate and graft biology are strictly coupled. SVF and its ADRCs (both terminally differentiated and progenitor pools) represent a complex milieu, consisting of a heteregeneous cell subpopulations, vast secretome, and possibly biologically active subcellular elements. These include preadypocites, stem cells and microvascular endothelial cells that have been shown to sustain preadipocyte viability in hypoxic conditions, thus promoting preadipocyte proliferation and differentiation. The ADRCs population contains endothelial progenitors cells which provide, along with tissue macrophages, a secretome of proangiogenic and anti-apoptotic growth factors[30]. SVF, for example, has been shown to hold factors such as IGF-1 which are involved with graft retention[17,30-33]. Nonetheless, there is still a second concern to be reviewed. It has been recently questioned the safety of "standard" fat grafting in patients with Ductal carcinoma in situ (DCIS). Such a concern may be even more substantial when considering a SVF enrichment with a considerable increase in stem cells and proangiogenic factors within the transplanted fat[23].

It has been documented that stem/progenitor cells from the SVF increase the motility and promote epithelial to mesenchymal transition of breast cancer cells[34,35], nevertheless the same SVF derived cells have been shown to inhibit the growth of numerous cancer cells[36]. The presence of protumorogenic and angiogenic factors in the graft requires a study of oncological safety. A number of anecdotal reports as well as some studies[37,38] have furthered the claim of cancerous cell proliferation when cultured in the presence of stromal vascular fraction cells. They suggest that placing stem cells in close proximity to an environment, that once harbored cancerous growth, may result in increased oncologic recurrence.

However, preclinical investigations remain controversial due to the models and cell types involved. On the other hand, Keramidas et al[39] report in humans shows interesting results about the inhibitory effect of mesenchymal stem cells on tumour growth[39]. A review of clinical studies[40] focused on breast fat grafting described oncological outcomes in more than 2,000 patients with no significative increase of new or recurrent cancers.

Despite the encouraging results obtained by Tissiani et al[41] reporting no loco-regional recurrences in patients undergoing SVF enriched fat grafts in secondary breast reconstruction, studies with a large number of patients and long-term follow up clinical data addressing this safety issue are currently lacking[41]. The cause of the debate on fat grafting safety is likely three-fold. First, some theoretical reasons (i.e., presence of protumorigenic factors within the graft) would suggest that fat grafting would influence cancer growth and metastasis. Second, nowadays the clinical researches on this topic have either small sample sizes and/or relatively short follow-up periods (i.e., the Perez-Cano trial, only 1 year). Last and more important, the results of laboratory studies have been interpreted beyond the context of their respective limitations; in fact, *in-vitro* culture system or preclinical animal model are not able to fully recapitulate the complexity of a unique clinical situation[42-53]. To our knowledge, this is the study with the largest series of patients that aims at tracking SVF-enrichment oncological safety over a long-term follow-up, reporting a single-center experience of SVF-en-

riched fat grafting in therapeutic NSM, in comparison to standard fat grafting and no fat graft control group[54,55]. The work showed no increase in rates of locoregional recurrence or systemic recurrence, supporting the oncologic safety of fat grafting and SVF-enhanced fat grafting in breast reconstruction.

As reported by many authors, SVF-FAT enhanced graft is approximately two times richer in stem/progenitor cells concentration than standard centrifuged fat graft, supporting our present series and previous experience with this procedure[56-62].

Despite the uniqueness of the enrolled populations and the long-term follow-up of the present series, this study displays some limits: first of all the absence of randomization, and second, a relatively limited number of cases, being a single-center series.

Conclusions

Local and systemic recurrence rates suggest that both centrifuged and SVF-enhanced fat reconstruction have a similar safety level in comparison to patients who did not undergo fat grafting in breast reconstruction after NSM. A large prospective, randomized, multi-center clinical study is still required to definitely assess the safety of fat grafting in a cancerous environment.

Conflict of Interest

None of the authors has any conflict of interest or financial interest in any of the products, devices, or drugs mentioned in this manuscript. None of the authors has any financial or personal relationships with other people or organizations that could inappropriately influence their work. The study followed the ethical standards of human experimentation, according to the Declaration of Helsinki.

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