Safety of Lipofilling in Breast Cancer Patients
Petit JY, Maisonneuve P, Rotmensz N, Bertolini F, Clough KB, Sarfati I, Gale KL, Macmillan D, Rey PC, Rietjens M

Keywords:
Lipofilling, fat transfer, breast cancer, mastectomy, breast conservative treatment, breast reconstruction, oncoplasty, recurrences,

Keys points:
• Biological considerations review of experimental research and translational studies
• Technique: differentiate the transfer technique with simple purification of the fat or the enrichment technique
• Clinical evaluation based on reliable statistical method to limit the risk of bias
• Randomized trial is the best method but not realistic in plastic surgery indications (patients refuse to be submitted to the surgeon choice)
• Prospective study is more reliable than retrospective but requires a long accrual period
• Prospective or retrospective should at least be case-control studies
• No definitive conclusions without large series, control group with a rigorous matching of the cancer criteria, and at least 5 y mean follow up
Introduction

Lipotransfer is a true technical revolution in plastic surgery and is increasingly used worldwide. Although known since several decades, the lipofilling has been indicated more recently in breast cancer patients to improve the results of breast reconstructions and to correct deformities after conservative treatment. A number of publications in plastic surgery literature underline the versatility of the technique and the quality of the results (1-8). They underline the efficacy of the lipofilling as a cosmetic procedure and consider it as a safe oncological only as a neutral biological material able to restore the body contour. Several studies underline the power of the transferred fat to regenerate the blood supply of the skin disorders after radiotherapy (9,10). Such active regeneration of the tissue can be explained by the presence of a high percentage of progenitor cells included in the fat tissue (11). Attention should be drawn to the recent and abundant preclinical studies which mention that adipose progenitor cells may promote breast cancer growth and metastasis. As recently shown, white adipose tissue (WAT)-derived progenitor cells can contribute to tumour vessels, pericytes and adipocytes, and were found to stimulate local and metastatic progression of breast cancer in several murine models (12-14). Experimental studies provide data on the endocrine, paracrine, and autocrine activity of the transplanted fat tissue (15). Adipocyte, pre-adipocyte and progenitor cells production of adipokines and several other secretions, can stimulate angiogenesis and growth of breast cancerous cells (16). The “tumour-stroma interaction” can potentially induce cancer reappearance by “fuelling” dormant breast cancer cells in tumour bed (17). Clinically, there is a case showing a local recurrence more than 13 years after apparent cure of an osteosarcoma, one year after a lipofilling of the shoulder for cosmetic repair (18). Moreover, a case-control study revealed a significant increase of local recurrences in intra-epithelial breast cancer patients who underwent a lipofilling procedure for breast reconstruction (19,20).

Concern about radiologic sequelae and surveillance difficulties by mammography due to lipofilling has led to an important literature. The risk of calcifications observed after lipofilling has provoked discussions concerning diagnosis of recurrences. This issue has largely been resolved by the distinction between true micro calcifications and macro calcifications related to fat necrosis as observed in most cases after fat transfer. Such images can easily be distinguished from suspicious calcifications (3,21,22).
In order to confirm the safety of lipofilling procedure in breast cancer patients, clinical studies based on adequate statistical method and accurate follow-up are required to demonstrate that the local recurrence rate as well as any cancer event is not increased in the fat grafted breast cancer patients.

**Biological considerations**

There is increasing evidence that obesity, an excess accumulation of adipose tissue occurring in mammals when caloric intake exceeds energy expenditure, is associated with an increased frequency and morbidity of several types of neoplastic diseases, including postmenopausal breast cancer. Disruption of the energy homeostasis results in obesity, inflammation, and alterations of adipokine signalling that may foster initiation and progression of cancer (23-25). Preclinical studies have suggested that differentiated cells of the white adipose tissue (WAT) and WAT-resident progenitors may also promote cancer growth and metastasis. We described that CD45−CD34+ progenitors from human WAT may promote breast cancer growth and metastases in preclinical models (13). Other recent studies, some of which are based on endogenous WAT expressing a transgenic reporter, showed a significant level of adipose cell contribution to tumour composition. However, WAT contains several distinct populations of progenitors, and these data were obtained using crude or mixed cell populations. We therefore decided to purify by sorting the two quantitatively most relevant populations of WAT progenitors (endothelial cells and adipose stromal cells; ASC) and to investigate in vitro and in vivo, their role in several orthotopic models of local and metastatic breast cancer. One study has recently described that EPCs are present in tissues other than the bone marrow, in particular in the adipose tissue of mice. Here, we report that human white adipose tissue (WAT) is a very rich reservoir of CD45-CD34+ EPCs (11). Compared with bone marrow–derived CD34+ cells mobilized in blood by granulocyte colony–stimulating factor (G-CSF), purified human WAT-derived CD34+ cells were found to express similar levels of stemness-related genes and significantly increased levels of angiogenesis-related genes and of FAP-α, a crucial suppressor of antitumor immunity (26). In vitro, WAT-CD34+ cells generated mature endothelial cells and endothelial tubes. In vivo, the coinjection of human WAT-CD34+ cells contributed to tumour vascularization orthotopic and significantly increased tumour growth.
and metastases in models of human breast cancer in non-obese diabetic severe combined immunodeficient (NOD/SCID) interleukin-2 receptor γ (IL-2Rγ)–null (NSG) mice.

Clinical evaluation

Technique of lipofilling

Two procedures should be differentiated according to the technique of lipofilling: the simple purification of the liposuction specimen and the adipose tissue-derived stromal cells (ADSCs) enrichment. The first one, so called “Coleman technique”, does not modify the concentration of ADSCs and the second, so-called the “enrichment technique”, increases the concentration of the ADSCs in the specimen which will be used for the reconstruction.

In the Coleman technique (27) the fat tissue is obtained by liposuction performed on a fatty area of the body (abdomen or thighs). The specimen is purified by a soft centrifugation to discard the oil and blood cells. Then the purified fat is injected in the area to be reshaped. Small differences of technique are proposed to purify the specimen without modifying the concentration of ADSCs. The main drawback of this technique is the frequency of reabsorption of the fat tissue injected in the following 6 months (28,29). Differently, the “enrichment technique divides the specimen obtained by liposuction in two parts. The first part is reserved for the final injection. The second one is processed in a machine using an enzymatic procedure (with a collagenase) to destroy the adult adipocytes and concentrate the ADSCs. The small amount of concentrated progenitor cells obtained by this procedure is then mixed to the first reserved specimen. Then the enriched fat tissue will be used for the reshaping of the breast (8). The promoters of the “enrichment technique” argue that the ADSCs concentration favours the regenerative process of the recipient tissues and decreases the risk of reabsorption of the fat tissue injected in the Coleman technique (30).

Clinical studies

We will focus the clinical discussion on clinical safety on three retrospective studies using the Coleman technique in breast cancer patients and comparing their results with those of a match control population.
In a first study, 321 patients receiving a lipofilling to improve the results of a reconstruction after partial or total mastectomy, were compared to a control group of 642 patients who did not receive any lipofilling. No statistical difference between the local recurrence rate of the two groups was observed.

The subgroup analysis did not find out any difference for the local recurrence rate according to the type of breast surgery (Breast conservative procedure or mastectomy), but revealed a difference between the two groups when considering only the intra epithelial cancer patients. Because of the small size of this group, no definitive conclusion was drawn (19).

To better investigate the local recurrence risk in intra epithelial cancer, we perform a new study focused only on a larger group of 59 intra epithelial breast cancers receiving a
lipofilling. This group was compared to a control group of 118 patients without lipofilling. Again, the local recurrence rate was significantly higher in the study group.

Although no clear reasons could explain such increased risk of recurrence in intra epithelial tumour, our results have been published to raise an alert to stimulate further studies with larger series and longer follow up (20).

These results have been compared with the results (unpublished) of Clough at the Institut du Sein in Paris. In a series of 48 intra-epithelial breast cancer patients treated with fat transfer, the authors observed 1.5% recurrence per year instead of 2.9% in the Milan
series. When we pooled the two series, we still observed a significant increase of LR with the controls of Milan series $P=0.0098$ while the difference for the Milan series alone was $P=0.0052$.

Results

1: The cumulative incidence of locoregional relapse is significantly higher in the lipofilling groups (Milan, Paris, Milan+Paris) than in the limited control group from Milan (individually matched 2 controls/lipofilling).

<table>
<thead>
<tr>
<th>Lipofiling</th>
<th>n</th>
<th>Events / Patient-year</th>
<th>Incidence per year</th>
</tr>
</thead>
<tbody>
<tr>
<td>MILAN</td>
<td>59</td>
<td>7/240</td>
<td>2.9%</td>
</tr>
<tr>
<td>PARIS</td>
<td>48</td>
<td>2/130</td>
<td>1.5%</td>
</tr>
<tr>
<td>MILAN+PARIS</td>
<td>107</td>
<td>9/370</td>
<td>2.4%</td>
</tr>
<tr>
<td>MILAN</td>
<td>118</td>
<td>4/674</td>
<td>0.6%</td>
</tr>
</tbody>
</table>
The difference is due to the particularly low rate of locoregional relapses (0.6% per year) in the control group.

A recent review (not published) of the Milan series of in situ patients with a longer follow up for the two groups does not find out a statistical difference as in the first study (Resultants Patrick).

MacMillan in Nottingham has performed a case control study with the same statistical methodology as our Milan study. Two hundred and eleven breast cancer patients receiving a lipofilling were compared with 422 controls. No statistical difference was observed between the recurrence rate of the study group and the control group: local (0.95% v 1.90%, p=0.33), regional (0.95% v 0%, p=0.16) and distant recurrences (3.32% v 2.61%, p=0.65). Among the 211 cases, 27 only were intra-epithelial and no locoregional recurrence was observed in this group. Macmillan concluded that “no evidence of increased oncological risk was associated with fat grafting in women previously treated for breast cancer.” (article in press)

Discussion

The three studies Milan, Paris, and Nottingham are homogenous in what concerns the technique of lipofilling all of them using the Coleman technique. MacMillan in Nottingham analysed his personal result after lipotransfer in breast cancer patients in a case-control study (article in press). He did not observe an increased number of local recurrences in the
in situ group as we observed in Milan and in Paris. But in his series, the number of in situ cases was probably too small to show a difference. However, the trend in the last Milan study is a disappearance of the significant difference between study group and the control group due to an increase of LR in the controls with a longer follow up. When we gather the cases of the three studies dealing with the Coleman technique, in order to get more statistical power, no more significant increase of LR is observed. The strength of the Milan studies and the Nottingham study is the quality of the comparison using precise match criteria. One of the difficulty to match the populations was the risk of bias due to the great variety of lapse time between the primary surgery (cancer treatment) and the date of the lipofilling. The study-population has been selected among the cases which did not have a cancer event during this lapse-time. The lipo-filled patients received their fat transfer at a different time after the cancer treatment and the length of the disease free period modifies the local recurrence risk after the lipofilling. Therefore, it was necessary to match the controls according to the length of the different lapse-time between primary surgery and lipofilling. The weakness of these studies is the population size of the intra-epithelial cancers: 59 patients in the Milan study and 27 in Nottingham study. The short follow up of the series was also an important critic: around two years after the lipofilling. The last review of the Milan study showed a disappearance of the significant difference.

Systematic review dedicated to the “Safety of Autologous Lipoaspirate Grafting in Breast Cancer Patients“, Krastev and collaborators analysed 394 articles dealing with fat transfer (31). After selection according to the content of cancer data, the quality of the follow up, the size of the series, the authors focused only 9 articles reaching the cancer criteria requirements. Among these 9 papers, they found no prospective study and no randomized trials. Only two retrospective studies were found to have a control group. One of Rigotti (9) compared the local recurrence rate of 133 mastectomy cases with lipofilling during the period pre and post lipofilling. No increase was observed and Rigotti concluded that lipofilling is a safe procedure in cancer patients aiming that if lipofilling was detrimental oncologically, the rate of recurrences in the second period post lipofilling should have been increased. Two critics have been made concerning the statistical methodology of the study (32). One was the exclusion of the breast conservative treatments which are patients with higher risk of cancer cells remaining in the breast after the treatment. The second deals with the methodology: the comparison between LR rate before and after the lipofilling could be reliable only if the actuarial rate of LR after the primary surgery was following a straight
line. It is usually stated that the rate of recurrence is higher in the first 5 years and then reach a plateau. Finally, Krastev concluded: “Whether lipoaspirate grafting promotes LRR in breast cancer patients is still unclear. To be able to answer this question, larger prospective trials with longer follow-up are needed.”

Other reviews concluded also that further prospective studies are needed to confirm the safety of lipofilling in breast cancer patients (33-36). Therefore, it will be extremely important to get more oncologic results after using the enrichment technique. The analysis of the literature showed that the progenitor cells should be responsible for the stimulation of remaining cancer cells, their concentration obtained thanks to the enrichment technique could stimulate the risk of cancer recurrences. We did not find reliable studies demonstrating the safety of enriched lipofilling in breast cancer patients. Although the “Restore study” published in 2012 did not find any recurrence in the series of 67 patients after a follow up of one year. The weakness of these results is the size of the study group, the lack of controls and the short follow up (37).
References


