ORIGINAL RESEARCH

A case controlled study of the oncological safety of fat grafting

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Abstract

Introduction

Fat grafting to the breast fulfils an increased clinical demand for a biocompatible filler in contour refinement, volume adjustment and tissue rejuvenation both in cosmetic and reconstructive procedures, and has been used for total breast reconstruction[1-5].

Whilst many clinical studies have reported on the efficacy of fat grafting for breast cancer patients in terms of its various indications[2,6-9], technical advancements[7,9-13,14], volume stability and graft survival[5,8,9,12, 14,15-19], radiologic safety[2,3,12,20-22,29,30], and complication rates[9,12,13,23,24,25,29,30], few studies focus on oncologic safety. Fewer still have adequate data and follow-up period to reach meaningful conclusions[9,19,20,26-30]. Others report outcomes for cosmetic breast patients only[5,24,31,32]. Where oncologic safety is assessed, many reviews focus on radiological sequellae interfering with mammographic surveillance[1,12,13,15,21].

Meta-analyses evaluating the oncologic safety of FG have begun to emerge in an effort to resolve this debate with a greater sample size[20,33-35]. However, there are limitations to the previous studies, particularly the consideration for confounding variables such as tumour histology, resection margins, receptor status and adjuvant treatments, which can only be corrected for by comparison with an appropriate control group[35-37].

Petit et al[39] reported the first case-matched retrospective series, (level 2b evidence). In this study we aimed to study a large series of breast cancer patients treated with FG in one institution using a matched cohort approach, and then systematically review relevant published cohort studies to which our results could be compared.

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Method

Between January 2007 and August 2013, 396 patients were treated with fat grafting (FG) to the breast at the Nottingham Breast Institute (NBI) for a variety of indications, including; breast asymmetry, contour deformity, correction of radiotherapy induced fibrosis and volume enhancement. Excluded from this study were; benign conditions (68), women whose primary oncologic surgery was performed elsewhere with missing cancer data (51), disease recurrence prior to fat grafting (35), and failure to identify a suitable case control match

(31). Data for 211 patients (DCIS = 27, Invasive carcinoma = 184) treated for breast cancer between 1977 and 2013 was included.

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For each of the 211 FG patients included in this study, two control subjects were matched from a prospective database of women who were treated for primary breast cancer at NBI and did not undergo FG intervention. Each control was matched for five variables; date of primary cancer operation (within 2 years), age (within 5 years), type of surgery, tumour histology, oestrogen receptor (ER) status and disease free interval by time of FG (Table 1). Further cancer variables were compared between the two populations to ensure homogeneity (Table 2 & 3). Similar to the Petit et al case-controlled series[34], the selected control patient had a disease-free period (*extrapolated* time A'-B') at least as long as the interval between oncologic surgery and FG procedure (time A-B) of the corresponding study patient (Figure 1). If the matched control had a recurrence prior to the end of this estimated time interval, then that patient was excluded, and another appropriate control was selected. The primary

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endpoint of the study was tumour recurrence, where the type and date were noted. The secondary endpoint was breast cancer-related death.

Data for women with recurrent disease prior to FG intervention (35) were collected for descriptive purposes and not included in the case-matched series, but were separately analysed. Patients treated for breast cancer at NBI were followed up annually with clinical examination and mammography for 2-5 years, depending on primary pathology.

Figure 1.

Fat grafting was performed according to the Coleman technique[7,16] without stem cell enhancement. Tumescence included 150mg laevobupivicaine in 1L 0.9% Normal Saline with 1: 1 000 000 adrenaline injected with a blunt cannula. Donor site selection was dependent on surgeon and patient preference, but was most

commonly the abdomen and upper thigh area. Fat was injected in thin strips. Some patients had more than one FG procedure with a mean of 1.28 per patient (range 1-4).

A systematic literature review included all studies with adequate descriptions of oncologic events and follow up, reporting on patients treated for breast cancer with subsequent fat grafting. Patients who had a recurrent event prior to FG intervention were excluded.

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Statistical method

The difference in prognostic variables between study and control groups was assessed using the chi-squared test. The main outcomes were recurrent oncological events in terms of local, regional and distant recurrences and death. Log Rank Kaplan-Meyer curves were used to calculate disease free survival (DFS).

Any event occurring simultaneously with a local relapse, such as a regional recurrence or synchronous metastases, was counted as a single event. In case of no events, the endpoint of the study was censored at the last follow-up. Statistical significance was considered at a probability of $p<0.05$. The impact of FG on risk of a recurrence was evaluated using the multivariate Cox proportion hazard regression model and expressed as hazard ratio (HR) with 95% confidence intervals (CIs).

Tables 1-4

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Results

Patient characteristics and follow-up are presented in Tables 1-4. The majority of patients in both the FG and control groups were treated by mastectomy; of these, almost 50% were skin sparing or nipple sparing mastectomies. Although the breast conserving surgery (BCS)

rate in the FG group was 16.6% (35 patients), this included only one patient with DCIS (0.7%) (Table 3).

Tables 2 & 3 show the distribution of non-matched variables in the FG and control groups for invasive cancer and DCIS. The populations are considered well matched, with Her-2 status (p=0.013) and Herceptin treatment (p=0.001) being the only different variables. This was most likely due to a large quantity of missing data in both groups as a result of non-routine Her-2 testing prior to 2005.

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Outcome analysis

The cumulative incidence of local recurrence in the FG and control groups was 0.95% and 1.90% respectively (p=0.744) (Tables 5&6). The locoregional recurrence (LRR) was equal between the FG and control groups (4/211, 1.9% and 8/422, 1.9%), at 0.7% per year (Table 6).

The characteristics of 4 women experiencing LRR events after the FG intervention are shown in Table 7.

Tables 5-7, Figures 2-3

Locoregional recurrence or distant metastases *prior* to fat grafting, (n=35)

The mean age was of patients in this cohort was 42 years (range 27-64) and mean follow-up after oncological surgery and fat grafting was 170 months and 30 months respectively, with mean time to baseline 140 months. There were 33 invasive cancers, and two cases of DCIS. Eleven patients (31%) initially had a mastectomy, but all patients eventually underwent mastectomy following a recurrence, so all patient had a mastectomy prior to fat grafting. Table 8 demonstrates the recurrent events prior to FG in this subgroup. Despite 40% of these patients having a previous ipsilateral local recurrence, no patient suffered further local event following FG

procedure. Three patients developed further non-local recurrent events (8.6%). One patient developed a palpable supraclavicular node 20 months after the FG intervention, one patient developed a distant metastasis 6 months after FG and one patient developed a new contralateral breast cancer.

Table 8

Comparison with Case-Controlled Series in the Literature

Table 9 compares this study series and the Petit[34] series. There were a significantly greater proportion of patients undergoing BCS in the Petit series compared to the current series (39% v 17%).

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Table 9

Systematic Review of Fat Grafting Case Series after Breast Cancer

Tables 10a & 10b

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Discussion

The results of this study show no significant association between fat grafting and disease recurrence in women previously treated for breast cancer. This was the case both for LRR (0.7% per year for both FG and control groups) and distant metastases (1.2% per year vs 0.9% per year for FG and control groups respectively). These rates are lower than those reported in the only other case-controlled series[38], where LRR was 1.9% per year in FG, 1.7% per year in controls and distant metastases 1.9% per year in both groups. This difference may be explained by the larger number of breast conserving surgery cases in their series.

Fat grafting after previous breast conserving surgery for breast cancer may be the best model for understanding the interaction between the fat graft and breast parenchyma in addressing the safety of fat grafting[37]. In our series, most of the indications for fat grafting

were in post-mastectomy patients, although half of these patients received a skin-sparing or nipple-sparing mastectomy. For breast conserving cases, the LRR was higher in the FG group compared to controls although not significantly so (2.1% vs 1.1% per year, p = 0.533). In the Petit multicenter series[30], comparable cumulative incidence curves were observed when BCS and mastectomy patients were analyzed separately. In their case-controlled series, Petit et al[38] reported a LRR of 2.2% per year in patients who had FG after BCS, which was not significantly different from controls.

For the mastectomy group in our series, there was no significant difference in LRR amongst FG and control groups; 0.4%per year vs 0.6% per year (p=0.848). Riggio[28] reported a LRR of 0.88% per year, Rigotti et al[26] a rate of 0.75% per year and Petit et al[38] a LRR of 1.4% per year for FG after mastectomy (Table 11b).

Unlike the Petit series[30,38,39], we observed no recurrences amongst patients treated with FG following surgery for DCIS. This could be

due to a number of factors. There was a long disease-free interval between primary oncologic surgery and FG in these patients (54 months), which is the critical window period for recurrences to occur. This may therefore be a select low risk group, although, a relatively short follow-up after FG (mean 32 months) was observed. Breast conserving surgery for DCIS was low for both the FG and control arms (0.5% & 1.7% respectively), although the rate of skin-sparing mastectomy (SSM) was 57.6% for FG and 49.0% for the control arms. In the Petit et al DCIS series[39], 6 recurrences occurred in FG patients and 3 in controls (5 year cumulative incidence 18% & 3% respectively). All recurrences in both groups had either BCS or some form of skin sparing mastectomy. Furthermore, the mean time interval between oncologic surgery and FG in their series was relatively short, at 25 months, and mean time to recurrence from FG was 12 months. The rate of positive or close margins in the FG group was 42% and 22% in the controls (p 0.38) and there was no specific information provided regarding re-excisions or adjuvant radiotherapy for these patients. Margin control and early FG intervention could be

factors in the high LRR observed in the FG group in this series involving DCIS patients[39]. In our series, all margins were clear.

In the Petit (DCIS) series[39], the likelihood of a recurrence was greatest if fat grafting occurred within 24 months of the primary oncological event. It is possible that early fat grafting may be associated with a greater risk of recurrence if performed in women with more risk factors for it. In this respect, our series may represent a low risk group. Ihrai et al[25] suggest a 36 month interval between primary surgery and fat grafting, and Riggio et al[28] 55 months. The mean time to fat grafting in our series and Petit et al[38] was similar (Table 10). The mean time to recurrence in the Petit DCIS series[39] was 12 months (range 5-24 months) and in the current series 50 months for invasive cancer (range 41-58 months).

There are limitations in interpreting individual studies reported in the literature. They are heterogeneous, retrospective, non-matched and many include cosmetic breast patients without exposure to breast

cancer. With respect to oncologic safety, it is important to focus on breast cancer patients only[35,36]. Case-matching supports the validity of the results[30,38]. Confounding variables such as resection margins, cancer histology, and receptor status can also directly influence the outcome. The FG and control groups in the current study are well matched (Tables 1-3).

However, case series may yield helpful information, if confounding variables are controlled for. Table 11a&b give a summary of studies presenting data for patients previously treated for invasive breast cancer or DCIS with FG. Our review of 1573 patients reported in the literature shows no evidence of an increased risk of breast cancer recurrence following fat grafting in terms of local (0.95% per year) or distant metastases (1.01% per year). Reassuringly, most series report both local and metastatic event rates between 1-2% per year. Petit et al[39] found a LRR of 3.2% per year in their case-controlled DCIS study which increased their overall LRR.

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Fat grafting is not a new technique, and has been exposed to criticism and controversy throughout its evolution[5, 42-47]. In 1987, a position paper released by the American Society of Plastic and Reconstructive Surgeons (ASPRS) Ad-Hoc Committee on New Procedures was 'unanimous in deploring the use of fat injection in breast augmentation' as its radiological sequalae would compromise the detection of breast cancer on surveillance mammography[48]. This issue has largely been resolved, as microcalcifications related to fat necrosis may be diagnosed after any type of breast cancer surgery and can be confidently distinguished from suspicious calcifications[21,22]. Despite the 'veil of silence' the ASPRS paper imposed, many surgeons were continuing to report on the use of FG for breast augmentation and reconstruction[5-9,12,14-16,18,21,23]. Coleman attempted to resolve these fears, by advocating meticulous planning, 'atraumatic liposuction', centrifugation and graft placement to provide 'pure, intact parcels of fat' to encourage integration and long-term graft survival[1,6,7,10,11,15,16]. He also became interested in the mechanisms of fat graft survival and stability, and postulated whether

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adipose-derived stems cells in the lipoaspirate may be involved, given their regenerative effect in experimental research and in 'replenishing natural tissues'[7,16]. Consequently, in a cautious public statement, both the American Society of Plastic Surgeons and American Society of Aesthetic Plastic Surgeons (ASPS and ASAPS) issued statements in 2007 that 'strongly support the ongoing research efforts that will establish the safety and efficacy of the procedure'[13,49,50]. |Similarly, the French Society of Plastic, Reconstructive and Aesthetic Surgeons (SOFCPRE)[51,52] and the UK NICE group[54] have issued caution with regard to FG after breast cancer. These societies acknowledged the lack of high level evidence in the literature demonstrating a link between fat grafting and breast cancer relapse. Based on recent reviews, the French have updated their recommendations[52], and a phase III multicentre randomized, controlled trial is currently taking place in France with the goal of investigating this issue[54].

Despite some concerns, it seems that FG is increasingly popular in breast surgery[4,55]. In a questionnaire to UK surgeons, 69% (48/70) of plastic surgeons and 11% (17/158) of breast surgeons are utilizing the technique (Ref). Most attitudes were positive with over 60% surgeons agreeing that the benefits of FG outweighed the risks. Similarly, the American College of Plastic Surgeons has reported 62% of their members regularly use FG for reconstructive breast surgery[4].

Fat is a metabolically active tissue consisting of a heterogeneous cell population secreting cytokines, hormones and growth factors[56-59]. A fat graft specimen contains mature adipocytes and preadipocytes, also known as adipose derived stem cells (ASC) [56,58,59]. ASCs have considerable angiogenic and antiapoptotic features and constitute 10% of the cell population, however graft survival is largely dependent on them given their huge proliferative contribution[14,40,57-59]. Adipocytes and ASCs release cytokines ('adipokines') to communicate with resident tissue for stimulating angiogenesis,

reducing apoptosis, and modulating the immune response during tissue repair[57,58,60,61]. The initial apprehension regarding FG and cancer resurgence came from obesity studies observing altered adipokine signaling in resident adipocytes that could facilitate cancer initiation and progression[62,62]. These 'adipokines' have been extensively studied, and include; leptin, adiponectin, resistin, metalloproteinase 11, hepatocyte growth factor (HGF), adipocyte-derived collagen VI, Interleukins, TGR-a, TGF-b, and VEGF[57,58,60-64]. Concern was raised that placement of these physiochemical factors at the site of a previous tumour microenvironment, may fuel the 'tumour-stromal interaction' through autocrine, paracrine and exocrine/endocrine that can result in tumour recurrence[14,56-61,64-66] and metastasis[58,67,68]. The direct influence of ASCs on residual or dormant tumour cells has been investigated with variable results. Co-cultures of human epithelial and adenocarcinoma tumour cell lines with MSCs in murine *in vitro*[58,67,69] and *in vivo* [66,68,69] models resulted in increased tumour cell viability, enhanced proliferation and reduced apoptosis of tumour cells. However, other groups found ASCs

capable of inhibiting proliferation, down-regulating cell signalling and abrogating tumour progression[70-72]. Zimmerman et al found that ASCs did not activate dormant cancer cells, but promoted residual active cancer cells into tumour growth and expansion. The group suggested that reconstructive therapy utilizing ASC-augmented whole fat should be postponed until there is no evidence of active disease and clear margins[69].

Local and systemic recurrence of breast cancer is observed at similar rates in our case controlled series, and may occur irrespective of the fat grafting intervention. Local recurrence and metastasis may be a manifestation of residual cancer stem cell activation and dissemination for reasons largely unknown, although the underlying molecular microenvironment may play a role[56,60,73]. Long term dormancy of cancer cells is a known phenomenon, but it is particularly evident in breast cancer patients in whom, even after 8 years of disease-free survival, a significant rate of late recurrence has been observed[74,75]. This suggests that many cancer types can

persist as ‘minimal residual disease’ and putative cancer stem cells can remain dormant for years, but are reactivated by still unknown mechanisms, often leading to rapid disease progression after a latent period[76,77].

The reason for the discordance between in vitro studies and clinical observations could be due to a number of reasons. The initial question of adipocytes secreting adipokines that could fuel cancer cell progression came from hypertrophied resident adipocytes in obese patients. This is different to the clinical setting regarding fat grafting, where normal adipocytes are transplanted into the breast in the mastectomy or prepectoral plane, and not into the parenchyma. In vitro and in vivo studies involve highly controlled microenvironments in constructed scaffolds or immunosuppressed animal models with many variables accounted for or eliminated. Furthermore, these studies have variable results with some adipokines stimulating cancer cell growth, and other suppressing it. The human subject is more complex, and there may be additional

unrecognized pathways that abrogate adipokine signaling after fat grafting into a previous cancer environment in the clinical setting.

As with any study, there are limitations with the current paper. This type of study would be difficult to design and run prospectively. The retrospective nature of this study is one drawback, but it facilitates adequate case matching. Case matching prospectively would be difficult due to the 5 variables requiring matching from entry into the study. If either of the two control patients had a recurrence prior to time B' (fat grafting intervention of the study patient), then they would need to be excluded, requiring that study patient also to be excluded, or two retrospective matches made at this point, rendering the study non-prospective in nature. The study is non-randomized, although randomization would be difficult due to the lack of a comparable alternative to fat grafting, and patients tend to know the benefits of the procedure and will ask for it, particularly if it enhances their reconstructive option. The ideal candidate to study the oncological safety of fat grafting is in the breast conserving patient. In our study,

there were low numbers of patients treated with BCS – around 16% in each arm (35 patients in FG group), however amongst our mastectomy group, around half of patients received either a skin-sparing or nipple-sparing mastectomy, with the small potential to leave breast tissue remaining (quote paper). There is controversy surrounding FG in patients with previous DCIS, particularly those treated with BCS, created by the Petit et al DCIS paper[39]. Our study cannot reliably contribute evidence to this question, as we had low numbers of DCIS patients treated with BCS.

However, despite these warnings from basic science, and our study's limitations, the current clinical study shows no evidence of increased oncological risk associated with fat grafting in women previously treated for breast cancer. This evidence should be interpreted with other similarly case controlled studies in establishing safe indications for fat grafting in this setting.

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