Effectiveness and Safety of Autologous Fat Transfer in Various Treatment Protocols

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Efficiëntie en Veiligheid van Autologe Vet Transplantatie voor Verschillende Behandel Protocollen

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“Effectiveness and Safety of Autologous Fat Transfer in Various Treatment Protocols”

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DISSERTATION

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by

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For Sahar

"Life is being one with you. Everything else is just waiting"

Contents

Chapter 1

General Introduction

Prelude

The nomenclature surrounding the technique of the reinjection of autologous fat has evolved parallel to its many other aspects during the formation of this dissertation. Therefore, please note that Chapters 2 to 6 adhere to the term "Autologous Fat Grafting" or AFG, whereas the Introduction as well as Chapters 7 to 11 use the definition "Autologous Fat Transfer" or AFT. Both terms are interchangeable in describing the same method.

Breast cancer and reconstructive options

Breast cancer is still the most common cancer in women in Europe [1,2] and worldwide with over nearly 1.7 million new cases diagnosed in 2012 [3]. In the Netherlands, this translates to one in eight women or 14.640 new cases each year [4]. Ongoing developments in early screening as well as better and more targeted therapies have dramatically improved the survival rate [5] with 5- and 10 year survival rates following diagnosis being 87% and 82% respectively [6]. One of the important distinctions that has to be made early in the diagnosis, for the treatment/survival as well as the reconstructive options, is the extension and the type of tumor. The two most common types of breast cancer are the ductal (originating from the epithelial cells of the milk ducts) and lobular (originating from mammary glandular tissue) carcinomas which can be contained to a local cluster of precancerous cells (carcinoma in situ or cis) or invasive in relation to adjacent tissues. Of the invasive or infiltrating type, ductal carcinoma comprises 70% to 80% compared to approximately 8% lobular carcinoma [7].

The most important goal in treating a patient with newly diagnosed breast cancer is survival. However, as survival has increased, esthetics become more important. Over the last decades smaller, less mutilating forms of breast cancer surgery have been developed that achieve the same survival rates as the rigorous radical mastectomies of the previous century. These types of breast surgery like lumpectomy, quadrantectomy, segmentectomy or partial mastectomy – followed by adjuvant radiotherapy – are collectively reffered to as "breast conserving therapy" (BCT) and have been shown to roughly equivalent mastectomy in long-term survival [8-10]. With this, the rate of unilateral skin-sparing-mastectomies (SSM) in the United States has been slowly declining since the

1990's while – with the discovery of BRCA 1 and 2 gen-mutations – the number of contralateral prophylactic mastectomies has been inclining [11].

Currently, there exists a large variety for reconstructive options following both BCT as well as SSM in which the decision making process is multifactorial and largely related to important factors like tumor size, -location and tumor-to-breast ratio. In general BCT is followed by some form of oncoplastic reconstruction according to the principles of *volume displacement* (i.e. oncoplastic reduction using Wise pattern) or *volume replacement* (i.e. anterior- or lateral intercostal artery perforator flap, thoraco-dorsal artery perforator flap, latissimus dorsi flap etc) [12-14]. These techniques do not only restore the direct postoperative breast volume and –contour but also decrease the possibility of long-term, post-radiation complications such as contour distortion and loss of volume due to parenchymal fibrosis and (scar) retraction [15,16]. Following a SSM the possibility for volume replacement techniques remain in the case of small breasts, but most often the patient has to choose between implant reconstruction and autologous reconstruction of which the current golden standard is the Deep Inferior Epigastric Artery Perforator (DIEP) flap [17].

While all these techniques can achieve excellent cosmetic results, they are not flawless or without (sometimes disastrous) complications. Whether it is a retracted scar or fibrosis following oncoplastic reconstruction, a capsule contracture or infected breast prosthesis necessitating removal or total flap loss following a DIEP flap, all contribute to the ongoing search for a superior, novel technique that can replace or complement the current repertoire of reconstructive options.

Breast augmentation

Interestingly enough, breast augmentation and autologous fat transfer (hereafter AFT) share the same history, with Czerny in 1895 describing transplanting a lipoma from the trunk to the breast in a patient deformed by partial mastectomy [18]. This was followed by the 1950s and 1960s when augmentation was carried out with solid alloplastic materials like, amongst others, polyurethane. Because of the significant complications, this technique was abandoned and replaced by the direct intra-parenchymal injection of semi-solid materials like beeswax, paraffin and even silicone itself before again being abandoned because of complications [19-21]. The development of the two types of implants that

are still being used today; the saline-filled- (mainly in the US) and the silicone gel-filled implants, started in the early 1960s [20,22]. Both implants share a silicone sheath, which, as part of the fifth generation, currently is being offered in a wide variety of sizes and shapes [23]. However, through the years the use of silicone implants has endured continuous scrutiny sometimes followed by scandals (PIP implants, 2010 [24]) which has led to the hypothesis of a possible link between silicone implants and various auto-immune- or connective tissue diseases. Multiple clinical studies [25-31] as well as a meta-analysis combining data from over 87 000 women [32] has shown no correlation between breast implants and auto-immune- or connective tissue disease. However, the recent discoveries of auto-immune/inflammatory syndrome induced by adjuvants (ASIA syndrome) [33] and breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) [34] have again spiked the clinical and scientific debate on the large scale use of silicone breast implants. With these new developments and the steady growth of women seeking breast augmentation (290.467 in the US in 2016 [35]) AFT is also currently being investigated as a viable alternative for implant based breast augmentation.

Scars

Besides the mutilating effects of breast cancer surgery, the sequelae of scars in general, regardless of the location or etiology can be equally emotional for patients [36]. Even though for physicians a scar, especially a matured scar signifies the endpoint of tissue healing, for patients its meaning can have pronounced implications for multiple aspects of daily live and, in addition, can be anchored on a psychological, social or even cultural level. The prevention and treatment of scars is a well-covered subject in present day education in plastic surgery. It covers patient selection (Asian population), surgical techniques and –scar revision, as well as treatment protocols containing pressure masks, silicone dressings, steroid injections, radio-/ cryotherapy, laser treatment and even antitumor or immunomodulatory drugs [37-40]. Given the gravity of some of these treatments it is understandable that the search for the superior scar treatment continues, and AFT have shown some promising results as will be discussed in Chapter 4,5.

Facial rejuvenation

For ages the face has been considered the most prominent feature of the human being and the motivation to alter its appearance or withstand the aging-process is as old as the work of Sushruta himself [41]. Soft-tissue fillers and facelifting are amongst the oldest known techniques used to alter the facial appearance, with Robert Gersuny injecting vaseline in 1899 [42] and Hollander performing the - self-proclaimed - first facelift in 1901 [43]. Similar to the history of breast augmentation, reconstruction of the face and AFT also share a common pioneer, with the German surgeon Gustav Neuber, in 1893, transplanting adipose tissue from the arm to correct a depressed facial scar following osteomyelitis [44]. Fast forwarding to 1992 when the first cosmetic use of the Clostridium Botulinum A exotoxin – better known as Botox – was described by Carruthers et al., for the treatment of glabellar rhytids [45] and we have concluded the three main pillars of modern facial rejuvenation.

Currently, there seems to be a shift towards the use of dermal fillers at the expense of surgical procedures like the facelift, which saw a 17% decrease since the beginning of the new millennium. On the contrary, Hyaluronic Acid and autologous "facial" fat transfer saw a 16.1% and 17% increase in 2016 respectively, compared with the previous year [46]. On one hand this shift might be representing the increasing demand of patients to achieve maximal results with minimal invasive – preferably outpatient clinic – procedures. On the other hand it might also represent the way our concepts of how the face changes with time, is evolving. The loss of elasticity that causes sagging of the skin was traditionally treated with resuspension and removal of excess (dermal) tissue. However, we know now that lack of support or volume beneath it might be the cause of sagging of the skin and can be – to some extent – treated with injectables before any surgical procedures [47].

Autologous fat transfer: General

As was previously stated, the genesis of AFT originated from the work of Neuber [44] and Czerny [18]. However, even back then the procedure was considered time consuming, difficult and above all unpredictable and with the absence of modern day technology, AFT was quickly discarded. It was Eugene Holländer [48,49] who, in 1909, suggested the idea of injecting fat through a cannula and both

Erich Lexer [50] and Charles Miller [51] further elaborated on the idea by describing its use for different conditions. However, the few favorable outcomes that were accomplished back then were largely overshadowed by, again, the unpredictable results as a consequence of the reabsorption rate and thus AFT fell out of favor. It wasn't until the work of Lyndon Peer [52], in the 1950s, that a better understanding began to develop about the reabsorption, which was considered around 45% at 1 year as a result of cell rupture/ -death influenced by adjustable factors like graft size and -handling. In the 1980s, with the advent of liposuction [53,54], there was a renewed interest in AFT and for the first time the preparation of the fat was considered an important contributing factor to its prognosis. In 1989 Chajchir et al. [55], were amongst the first to deliver recommendations based on personal experience but it was Sydney Coleman [56-58] who first standardized the technique in 1990, which is more or less in the same manner, still used today.

Autologous fat transfer: Technique

The first standardized AFT technique, also named the Coleman Method or structural fat grafting, involved (1) infiltration of the (previously selected) harvest-location with a local anesthetic solution, (2) followed by the gentle harvesting of fat, (3) centrifugation to remove nonviable components and provide predictable volume, and (4) reinjection of the fat in small aliquots to increase the surface area, therefore providing better blood supply to the grafted tissue [59,60]. Even though the Coleman Method has reached its silver jubilee, it is to this day the most clinically practiced AFT technique, apart from small variations in certain aspects like cannula size and centrifugation time. However, at the same time, the quest for improvement of the adipocyte yield and fat survival has instigated an enormous spike in new methods for every aspect of the AFT process.

In clinical practice the choice of harvest-location is generally based on the desires of the patient and the accessibility of the fat. Several studies have indicated superiority of one harvest location over the other with Jurgens et al. [61] preferring the abdomen over the hip/ thigh region when considering the yield of SVF and ADSCs (see further). However, Saint-Cyr et al. [62] found better volume retention of trochanteric harvested fat which they attributed to higher numbers of adipocytes and so-called colony forming units (CFUs). With this, it seems like the golden standard for the harvesting location is yet to be found, as was reported in the recent review by Strong et al. [63]. However, both the harvest- as well as the

infiltration cannulas do have a great influence on the graft take since mechanical aspects – amongst others – have been proven to affect the viability of both the adipocytes (20% of lipoaspirate [64-66]) as well as the remaining cells, summarily called stromal vascular fraction (SVF) [67-69]. The infiltration solution has been standardized in various protocols which led to specially named solutions like the Coleman- and Klein solutions but generally comprise of saline plus a local anesthetic for postoperative pain relief, combined with epinephrine for vasoconstriction. A recent study, however, showed that not the contents of the solution, but rather the osmolality might influence the viability of both the adipocytes as well as the SVF [70].

Preparation of the lipoaspirate – one of the most debated subjects – in the clinical setting, besides centrifugation, generally consists of decantation or filtering through a simple membrane (mesh) eventually combined with washing. All these forms of preparation have been reported as superior over each other, in various studies. For example, decantation, which describes the process of settling the precipitate and subsequently distracting the required (middle) layer, showed the highest number of adipocytes and mesenchymal stem cells compared to washing and centrifugation in one study [71]. However, centrifugation has proved itself superior over the other methods on important aspects like cell concentration/ -viability and subsequently "graft absorption rate" in a number of different studies [72-75] with ideal settings generally not exceeding either 3000 rpm or 2 minutes. While no consensus on the golden standard in preparation has been reached there seems to be a clinical preference for centrifugation, possibly also because of the adherence of most surgeons to the original Coleman Method.

A second much debated subject regarding AFT technique are the graft take enhancing methods which can be divided in either (1) pre-/ postoperative external volume enhancing techniques like the Breast Enhancing and Shaping System (BRAVA®) or perioperative techniques like performing rigottomies (sharp adhesiolysis prior to fat grafting) and (2) AFT supplementation. Both rigottomies, named after its inventor Dr Rigotti [76,77], as well as pre- and postoperative use of BRAVA®, popularized by Dr Khouri [78,79] have shown promising results. Supplementation describes the process of enriching the prepared fat with autologous material, mainly derived from the previously mentioned SVF or, in some cases, venous blood. A large variety of different supplementation protocols have been reported on and the nomenclature herein is substantial, but what it comes down to is that the SVF or venous blood is prepared parallel to

the preparation of the grafted fat and additionally added to increase the graft take. In the case of SVF the preparation is generally designed to extract the adipose-tissue-derived-stem-cells (ADSCs) which have shown the ability to differentiate into adipocytes – amongst other cell lines – in multiple studies [80,81]. When venous blood is used for supplementation it is commonly for extraction of either the Platelet-Rich-Plasma (PrP) or the Platelet-Rich-Fibrin (PrF) [82] which is described to improve either the angiogenesis [83,84] or the vascularization of the graft respectively [85-87].

Finally the injection technique and -planes are a subject of great scientific interest with substantial variations related to not only indication but also comorbidities and previous surgery. While there seems consensus on the technique - which should be in a retrograde, fanning matter, leaving small aliquots of fat [88,89] – the injection planes, especially following, breast surgery can be of significant importance. This importance derives, amongst others, from the possibility of remaining (dormant) tumor cells following breast cancer surgery, which hypothetically can progress and become carcinogenic due to ADSCs related hormones [90].

Aim

The aim of this thesis is to give a clear overview of the technique, effectiveness and safety of AFT and its clinical application as a substitute or additive in (1) breast reconstruction, (2) breast augmentation, (3) facial rejuvenation and (4) the treatment of (retracted) scars.

The following research questions are addressed:

- What is the effectiveness and safety of AFT in addition to breast reconstruction/ and –augmentation in terms of; oncological recurrence, radiological follow-up, complications, volume retention and patient-/ surgeon satisfaction (**Chapter 2, 3)**
- What is the effectiveness of AFT in the treatment of scar related conditions, such as; appearance and skin characteristics, restoration of volume and/or (three-dimensional) contour, itch, and pain (**Chapter 4, 5**)
- In the absence of a conclusive and definitive answer on the oncological safety of AFT after breast cancer surgery, can we give an up-to-date,

comprehensive overview of both the basic science and the clinical studies regarding the subject (**Chapter 6**)

- What is the current clinical status in Europe regarding the AFT technique practiced and the performing surgeons background, -experience and -opinion (**Chapter 7**)
- In terms of AFT effectiveness; what is the difference between the satisfaction of different groups of patients and physicians, and can we quantify this difference for various indications in breast surgery (**Chapter 8**)
- What is the effectiveness and safety of the solitary use of AFT for facial rejuvenation, in terms of; complications, volume retention and patient-/ surgeon satisfaction (**Chapter 9**)

Outline of this thesis

AFT is a topic that – over the last two decades - has contracted a great deal of scientific interest for multiple indications. In plastic and reconstructive surgery this interest is generally focused on its application in the treatment of Dupuytren disease [91] and its abilities to correct contour deformities because of its volume-restoring capabilities. The part of the human body where the plastic surgeon is most often confronted with problems regarding contour deformities and a general lack of volume are the breasts, mostly of course in the female population. Therefore, in **Chapter 2 and 3**, important features of AFT in breast reconstruction and breast augmentation respectively are reported on by way of a systematic review with meta-analysis of important aspects like complications, satisfaction and oncological safety.

Scars are universal in the sense that no human being can go throughout life without suffering a few of his-/ or her own. However, at times scars can become a source of debilitating functional and emotional sorrow, let alone physical pain and can therefore be filed under contour deformities when discussing the therapeutic options of AFT. Henceforth, in **Chapter 4** we report on the effectiveness of AFT in the treatment of important scar related conditions like; appearance, restoration of volume and/or (three-dimensional) contour, itch, and pain by way of a systematic review of the recent and relevant literature. In **Chapter 5** we elaborate on this by way of a letter to the editor, in which we further highlight its use for specific pain syndromes like the post-mastectomy pain syndrome as

well as the mechanisms through which scars and fibrotic tissue is believed to soften.

Following up on the oncological safety previously discussed in chapter 2, we continue our search for an answer regarding this highly debated topic in **Chapter 6**. Herein, we give a tabulated overview of both the basic science studies as well as the clinical studies that report on either the carcinogenic or tumor-suppressive properties of AFT in relation to breast cancer.

As was previously mentioned, the AFT technique varies widely, while simultaneously, reported scientific data derives mainly from a small percentage of practitioners working in high-volume centers. Therefore, in **Chapter 7** we report on an AFT survey study, highlighting important AFT technical aspects like cannula sizes and methods of preparation, currently used in everyday clinics in 10 European countries. Furthermore, the opinion of the 358 respondents, on important topics like expected patient satisfaction and (cause of) volume retention is reported.

The majority of clinical studies today, report on patient- and surgeon satisfaction rates but comparisons in the cosmetic appreciation of the procedure, between groups of patients and physicians, based on background and experience has not been thoroughly studied. Therefore, in **Chapter 8**, the interrater agreement between different groups of patients and physicians in the cosmetic evaluation of AFT for various indications – following breast surgery - is discussed.

And finally, with the increasing demand for less invasive methods for facial rejuvenation and the upcoming use of AFT as “the” promising next generation dermal filler for the face, we systematically reviewed the most important properties of AFT for this purpose in **Chapter 9**.

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Chapter 2

Autologous Fat Grafting after Onco-Plastic Breast Reconstruction; A Systematic Review and Meta-Analysis on Oncological and Radiological safety, Complications, Volume Retention and Patient/ Surgeon Satisfaction

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Abstract

Objective: This study presents an up-to-date overview of the literature on autologous fat grafting (AFG) in onco-plastic breast reconstruction, with respect to complications, oncological and radiological safety, volume retention and patient/surgeon satisfaction.

Background: Although AFG is increasingly being applied in onco-plastic breast reconstruction, a comprehensive overview of the available evidence for this procedure is still lacking.

Methods: A systematic review following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement was conducted. Case series, cohort studies and randomized controlled trials (RCTs) reporting on relevant outcomes of breast reconstruction with supplemental AFG were included.

Results: In total, 43 studies were included reporting on 6260 patients with a follow-up period ranging from 12 to 136 months. The average locoregional and distant oncological recurrence rates after breast reconstruction with AFG were 2.5% (95% confidence interval (CI) 1.7-3.7) and 2.0% (95% CI 1.1-3.5), respectively. Fewer cysts and calcifications were seen on radiological images for this procedure than for other types of breast surgery. However, more biopsies were performed based on radiological findings (3.7% vs. 1.6%), and more cases of fat necrosis (9.0% vs 4.7%) were seen after treatment with AFG. The total complication rate of 8.4% (95% CI 7.6-9.1) is lower than those reported following other reconstructive breast procedures. The mean volume retention was 76.8% (range 44.7-82.6%) with a satisfaction rate of 93.4% for patients and 90.1% for surgeons.

Conclusions: AFG in breast reconstruction is a promising technique. Safety is not compromised as cancer recurrence and complications are not observed. Whether AFG interferes with radiological follow-up remains to be elucidated. Randomized trials with sound methodology are needed to confirm these conclusions.

Introduction

Autologous fat grafting (AFG) was first introduced in the 1890s by Neuber and Czerny, who began to transplant fat tissue and lipomas. Since then, interest in the technique waned because of the high reabsorption rates [1]. In 1987, AFG was prohibited in breast reconstruction procedures, as its use was found to impede cancer diagnostics as well as possibly stimulate the formation of breast cancer itself [2]. After Coleman [3,4] standardized the procedure in 1995 and achieved greater procedure accuracy and good results, further studies led to the 2009 statement by the Fat Graft Task Force of the ASPS that 'the procedure is not prohibited (due to the lack of evidence) nor recommended, and should only be performed by specialized surgeons [5]. Since then, AFG has been increasingly used in reconstructive breast surgery. The safety and efficacy of AFG in breast surgery, as well as other indications, are currently of great interest, with several original studies and reviews being published. However, the latter mainly include case reports or small case series, and they generally focus on one or two outcomes. As this technique becomes more widely accepted, more questions arise, as indicated by the editorial piece of Longaker et al. [6]. Hence, our aim is to present a comprehensive overview of the current evidence on the outcomes of onco-plastic breast reconstruction with the (supplemental) use of AFG and to reveal gaps in the current literature to form a basis for further research.

Specifically, we aim to determine the following:

1) Oncological safety: the frequency of oncological recurrences in relation to the type of malignancy.
2) Radiological safety: the type and frequency of radiological findings and the number of biopsies based on these findings.
3) Complications: the frequency and type of complications in relation to the graft technique used.
4) Fat grafting technique: the number of grafting procedures and the volume of grafted fat.
5) Efficacy: patient/surgeon satisfaction and fat graft retention in relation to adjuvant radiotherapy.

Methods

A systematic review of the literature on AFG in the female breast was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (www.prisma-statement.org) [7]. PubMed, Embase.com, Wiley/Cochrane Library and Web of Science were searched from inception (by JG and JCFK) between January 1996 and November 2014. The following terms were used (including synonyms and closely related words) as index terms or free-text words: 'fat' or 'adipocyte' or 'lipo' and 'grafting' or 'filling' or 'transplant'. The full search strategies for all the databases can be found in the Supplementary Information. Studies that were considered potentially relevant based on the titles were stored using the RefWorks database, with no restriction on language, type of study or publication media. Bibliographies of retrieved studies were manually searched for relevant and possibly missed references.

Eligibility criteria

Original studies on the application of fat grafting (with or without supplementation) in breast reconstructive procedures after breast cancer surgery in women were considered eligible for inclusion. The studies were collected by two independent reviewers (JG, VN) and screened on outcomes such as complications, radiological appearances, oncological safety (i.e., recurrence rate) and volume retention or patient/surgeon satisfaction. Duplicate studies, case reports or case series with a sample size of <10 and studies with a mean follow-up period of <12 months were excluded.

Study selection

The abstracts of selected studies were evaluated independently by two researchers (JG and VN). When found eligible, the full-text article was retrieved for evaluation, data extraction and inclusion in the systematic review. Discrepancies between the two reviewers were discussed; when a solution was not found, a third reviewer (MG) was consulted. When a study could not be retrieved from the electronic media or the local library, the authors were contacted to request a copy.

Data collection process

Data were extracted by one researcher (JG) using standardized tables and were then checked by a second reviewer (VN). The following data were extracted from each article: authors, date of publication, number of subjects, indication for the procedure, type of study, technique used for adipocyte implantation, follow-up time, efficacy of treatment, patient satisfaction, clinical complications, volume retention, radiographic changes and incidence of primary and recurrent breast cancer. Randomized controlled trials (RCTs), prospective and retrospective observational or comparative cohort studies and case series with sufficient sample size and follow-up were evaluated for the following factors: clear description of inclusion and exclusion criteria, method of patient selection for the procedure (i.e., consecutive vs. nonconsecutive recruitment), adequate sample size (at least 10 patients), use of objective outcomes and sufficient duration of follow-up (1 year). The included studies were assigned a level of evidence according to the Oxford Centre for Evidence-Based Medicine (2011) by two independent reviewers (JG and VN). Discrepancies in scoring were discussed by all reviewers. The principal summary measures are means over follow-up periods and percentages with the actual number given between parentheses.

Statistical analysis

To analyze the oncological safety, a random-effects meta-analysis was performed to account for the heterogeneity among the different studies. For the analyses of the complications and radiological findings, the data were pooled to calculate the overall proportion with a 95% confidence interval. Due to insufficient data, statistical analyses of the fat grafting technique, volume retention and patient and surgeon satisfaction could not be performed.

Risk of bias across studies

Observational studies and clinical trials without detailed randomization protocols were deemed studies with a high risk of bias.

Results

After screening, a total of 44 studies were included (Figure 1) [8-51]. Two studies [12,13] described the same group of patients; thus, one of the studies [13] was excluded from the analyses, leaving 43 studies. The included studies were published in the period 2005-2014. This included almost equal numbers of retrospective and prospective cohort designs (20 vs. 21, respectively), as well as two clinical trials (level II OCEBM). Eight level III studies and 33 level IV studies were noted, and overall they involved a total of 6260 patients. The mean follow-up was 33.2 months (range: 12-136). Extracted data are summarized in Tables 1-7.

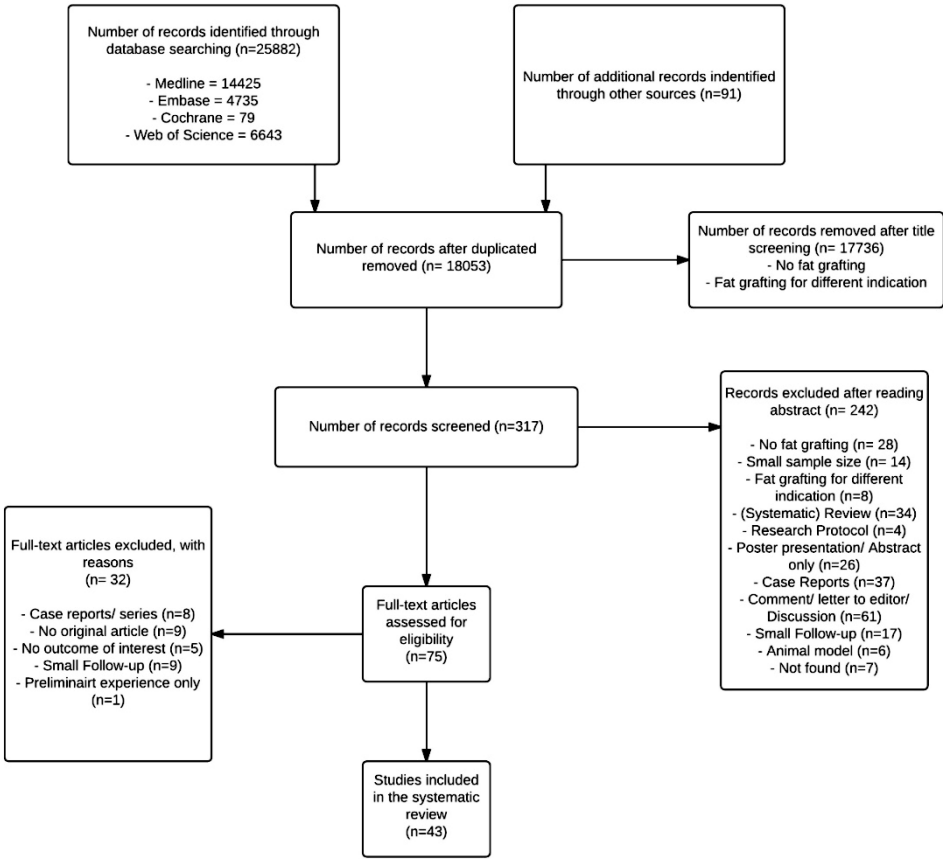


Figure 1: Flowchart of included studies

Indication

In 28 studies, the indication and the previous form of surgical breast cancer treatment were reported [8,9,14,17-32,37-39,41,44-47,49]. Twenty-nine studies commented on the form of reconstruction in addition to AFG [8-10,12,16-20,22-25,27,29,31-33,37,39,41,42,44-50]. Mastectomy (MST) was performed in 1840 of 2610 breasts and conserving therapy in 837. No preventive mastectomies were reported. Isolated AFG treatment was performed in 1094 breasts, whereas AFG was combined with an unspecified form of reconstruction in 1102 cases. AFG was combined with implant reconstruction in 709 breasts, and with tissue expander breast reconstruction in 285 breasts. AFG was also combined with autologous flap reconstructions. The latissimus dorsi (LD) flap was used most frequently with 283 reported cases, whereas the transverse rectus abdominis myocutaneous (TRAM) flap was used in 90 breasts. The form of flap reconstruction was unspecified in 207 breasts.

Table 1: Baseline: Characteristics of included Studies

Reference	Year	Study design	Total	Reconstruction	Follow up: months (mean/ median/ actual)	Age: years (mean/ median)	Reported outcomes	Level of Evidence (OCEBM))
Pierrefeu-Lagrange[16]	2006	Prospective cohort	30	30 (34)	12 (actual)	51 (mean)	RS, OS	IV
Missana [44]	2007	Prospective cohort	69	69 (74)	11.7 (mean)	51 (mean)	C, RS	IV
Delay [8]	2008	Prospective cohort	42	42 (42)	31.2 (mean)	50.7 (mean)	C, PS, OS	IV
Gosset [11]	2008	Prospective cohort	21	21 (21)	31.2 (mean)	50.7 (mean)	RS	IV
Panettiere [46]	2009	Prospective case control	20	20 (20)	17.6 (mean)	49.1 (mean)	C	III
Delay [17]	2009	Retrospective cohort	880	850 (850)	-120	/	RS, OS, C	IV
Illouz [10]	2009	Prospective cohort	820	435	136 (mean)	45.6 (mean)	C, RS	IV
Sinna [33]	2010	Retrospective cohort	200	200 (200)	14.5 (median)	48.7 (median)	PS, C	IV
Serra-Renom [47]	2010	Prospective cohort	65	65 (65)	12 (mean)	Range only: 34-62	C	IV
Rigotti [29]	2010	Prospective cohort	137	137	76.8 (mean)	46.5 (median)	OS	IV
Rietjens [18]	2011	Retrospective cohort	158	157 (191)	18.3 (mean)	48 (mean)	RS, OS, C	IV
Petit [19]	2011	Retrospective cohort	513	513 (646)	19.2 (mean)	52.1 (mean)	RS, OS, C	IV
de Blacam [48]	2011	Retrospective cohort	49	49 (68)	28.8 (mean)	47.4 (median)	C	IV
Sarfati [37]	2011	Prospective cohort	28	28	17 (mean)	45 (mean)	C, OS, PS	IV
Beck [41]	2011	Prospective cohort	10	10	- 36	49 (mean)	PS, VR, RS, C	IV
Petit [28]	2012	Retrospective matched cohort	321	321 (321)	26 (median)	45 (median)	OS	III
Salgarello [49]	2012	Retrospective cohort	16	16 (21)	15(mean)	41 (mean)	PS, C	IV
Seth [20]	2012	Retrospective cohort	69	69 (90)	24.8 (mean)	49.4 (mean)	C, OS	III
Doren [25]	2012	Retrospective cohort	278	278 (468)	28 (median)	51 (mean)	PS, OS	IV
Perez-Cano[9]	2012	Prospective clinical trial	67	67 (68)	12 (actual)	52 (range 37-68)	OS, RS, C	II
Petit [26]	2013	Retrospective cohort	59	59 (59)	38/42 (median)	49 (median) / 50 (median)	OS	III
Gentile [12]	2013	Retrospective cohort	100	65 (65)	12 (actual)	Range only: 19-60	VR, C, RS	III

Reference	Year	Study design	Total	Reconstruction	Follow up: months (mean/ median/ actual)	Age: years (mean/ median)	Reported outcomes	Level of Evidence (OCEBM))
Delay [42]	2013	Retrospective cohort	31	31 (36)	78 (mean)	23 (mean)	PS, C	IV
Ho Quoc [51]	2013	Retrospective cohort	1000	1000 (1000)	54 (mean)	39 (mean)	C	IV
Constantini [32]	2013	Prospective cohort	24	24 (24)	12 (actual)	50.8 (mean)	RS, OS	IV
Sarfati [27]	2013	Prospective cohort	68	68 (68)	23 (mean)	46 (mean)	C, OS, PS	IV
Riggio [23]	2013	Prospective cohort	60	60 (60)	90 (median)	49 (mean)	OS	III
Ihrai [24]	2013	Retrospective cohort	64	64 (64)	46 (mean)	/	OS, RS, C	IV
Hoppe [22]	2013	Retrospective cohort	28	28 (35)	31.2 (mean)	52.4 (mean) 3	C, PS	IV
Thekkinkattil [50]	2013	Retrospective cohort	10	10 (10)	15/40 (mean)	56 (mean)	PS, C, RS	IV
Fiaschetti [15]	2013	Retrospective cohort	15	10 (13)	12 (actual)	46.27 (mean)	VR	IV
Derder [43]	2014	Retrospective cohort	10	10 (13)	68 (mean)	17.5 (mean)	RS, PS, VR, C	IV
Del Vecchio [40]	2014	Prospective cohort	30	3 (3)	12 (mean)	/	VR	IV
Longo [38]	2014	Prospective comparative cohort	21	21 (29)	34.8 (mean) 17.2 (mean)	36.64 (mean)/ 38.70 (mean)	OS	III
Kim [39]	2014	Retrospective review	102	102 (102)	28.7 (mean)	46.3 (mean)	OS, RS, C	IV
Hitier [21]	2014	Retrospective cohort	150	150 (150)	12 (actual)	50.7 (mean)	C, PS	IV
Semprini [30]	2014	Retrospective cohort	151	151 (151)	45 (mean)	Range only: 40-72	OS	IV
Molto-Garcia [14]	2014	Prospective cohort	37	37 (37)	12 (actual)	55 (mean)	C, OS, PS	IV
Mestak [45]	2014	RCT	30	30 (30)	21 (mean)	38.3 (mean)	PS, C, RS	II
Brenelli [31]	2014	Prospective cohort	59	59 (59)	34.4 (mean)	50 (mean)	OS, C, RS	III
Chiu [34]	2014	Prospective cohort	282	-	23.7/23 (mean)	34.9 (mean) / 31.2 (mean)	PS, VR, C	IV
Li [36]	2014	Prospective cohort	105	2	18 (mean)	31.3 (mean)	RS, OS, C	IV
Ho-Quoc [35]	2014	Prospective cohort	31	31 (34)	72 (mean)	21 (mean)	PS, C	IV

Abbreviations: Volume Retention (VR), Patient Satisfaction (PS), Radiological Safety (RS), Oncological safety (OS), Complications (C), OCEBM (Oxford Centre for Evidence-Based Medicine).

Oncological safety

Twenty-one studies reported on oncological safety after cancer treatment and breast reconstruction with AFG in a total of 3020 patients (Table 2) [8,9,14,16-20,23-32,37-39]. Two studies [16,17] did not present this information, and one [18] only specified the type of oncological surgery in relation to the number of operated breasts. In 14 studies, 1371 invasive carcinomas and 512 carcinomas in situ were reported [8,14,19,20,23-26,28-31,37,39]. These included 569 cases of ductal carcinoma, 35 cases of lobular carcinoma, two medullary carcinomas and two mixed forms (ductal and lobular). Three cases of phyllodes, nine fibroadenomas and one case of Paget's disease were also noted. In their study, Perez-Cano et al. reported no local recurrence, but a new case of bone metastasis in one patient was considered as natural progression of the disease. Due to this aberrant definition of 'recurrence', this study was excluded from the analysis [9]. Meta-analysis over the total cohort of patients showed an LRR of 2.5% (95% confidence interval (CI) 1.7-3.7) and a DR of 2.0% (95% CI 1.1-3.5) with no difference between MST and breast-conserving therapy (BCT) patients ($p = 0.69$). Only three studies [23,24,26] ($n = 183$ patients) reported on the histological type of the recurrent tumour (nine ductal and one lobular) – all locoregional and all of the same histological type as the primary tumour.

Table 2: Oncological safety: Overview of type of oncological- and reconstruction surgery, type of carcinoma, inclusion of radio-/ chemo- and/or hormonal therapy and locoregional-/ distant cancer recurrence rates.

Reference	Year	Study population characteristics								Relevant outcomes			Level of Evidence
		# pat.	Mean age	Initial surgery	Invasive carcinomas	Carcinomas in situ	RT	CT	HT	Reconstructive surgery	Mean follow up (months)	Locoregional recurrence	Distant recurrence	
Pierrefeu-Lagrange [16]	2006	30	51	NR	NR	NR	NR	NR	NR	LD	12	0/30	0/30	IV
Delay [8]	2008	42	50.7	BCT	39/42	3/42	36/42	21/42	9/42	NR	31.2	1/42	5/42	IV
Delay [17]	2009	734		NR	NR	NR	NR	NR	NR	NR	-120	0/734	0/734	IV
Rigotti [29]	2010	137	/	mMST	105/137	31/137	22/137	NR	NR	NR	76.8	5/137	0/137	IV
Rietjens [18]	2011	158/ 191b	48	MST 114/191, BCT 77/191	NR	NR	96/158	NR	NR	Pr 115/191, LD 6/191, TRAM 3/191, TRAM + Pr 2/191	18.3	1/158	0/158	IV
Petit [19]	2011	513	52.1	MST 370/513, BCT 143/513	405/513	108/513 (101 duct, 7 lob)	395/513	NR	NR	Pr 238/513, Flap NS 135/513, Pr + Flap NS 9/513	19.2	13/513	16/513	IV
Sarfati [37]	2011	28	45	MST 28/28	28/28	0/28	28/28	NR	NR	Pr 28/28	17	0/28	0/28[a]	IV
Petit [28]	2012	321	/	MST 196/321, BCT 125/321	284/321	37/321, DIN 35/321 LIN 2/321	NR	NR	NR	NR	45.3	8/321	5/321	IV
Perez-Cano[9]	2012	67	52	BCT 67	NR	NR	61	NR	NR	NR	12	0/67	NR[k]	II
Petit [26]	2013	59	/	MST 47/59, BCT 12/59	0/59	DIN 57/59, LIN 2/59	7/59	NR	28/59	NR	38 (median)	6/59	0/59	III
Constantini [32]	2012	22	50.8	MST 14/22, BCT 8/22	NR	NR	15/22	NR	NR	Pr 8/22, DIEP 4/22	12	1/22	0/22	IV
Seth [20]	2012	69/ 90b	49.4	MST 69/69	50/90[c]	17/90	prO 2/90 poO 74/90	NR	NR	Flap NS + ITER + Pr 69/69	24.8	0/69	0/69	IV
Sarfati [27]	2013	68	46	MST 68/68	NR	NR	68/68	NR	NR	Pr[d] 68/68	23	0/68	3/68[e]	IV
Doren [25]	2012	278[f] 448b	51	MST 278/278	94/223	129/223	prO 56/278 poO 34/278	78/278	NR	TE 280/448, TRAM 69/448, LD + TE 72/448, LD + Pr 2/448, Pr 14/448 DIEP 10/448	56.2	6/278	3/278[g]	IV
Riggio [23]	2013	60	49	MST 60/60	53/60	5/60[h]	NR	NR	NR	Pr 49/60, Flap NS 10/60	90 (median)	2/60	5/60	III

Reference	Year	Study population characteristics								Relevant outcomes			Level of Evidence
		# pat.	Mean age	Initial surgery	Invasive carcinomas	Carcinomas in situ	RT	CT	HT	Reconstructive surgery	Mean follow up (months)	Locoregional recurrence	Distant recurrence	
Ihrai [24]	2013	64	/	MST 50/64, BCT 14/64	51/60	10/61[i]	NR	NR	NR	Pr 33/64, LD + Pr 17/64	46	2/64	3/64	IV
Longo [38]	2014	21	36.64 38.70	nsMST 21/21	NR	NR	10/21	NR	NR	NR	34.8 17.2	0/21	0/21	III
Kim[39]	2014	102	46.3	MST 102/102	60/102	42/102	NR	NR	NR	LD 41/102, TRAM 15/102 Pr 46/102	28.7	1/102	0/102	IV
Semprini [30]	2014	151	/	BCT 151/151	115/151	25/151[j]	151/151	NR	NR	NR	45	0/151	0/151	IV
Molto-Garcia [14]	2014	37	55	BCT 37/37	35/37	2/37	NR	NR	NR	NR	12	0/37	0/37	IV
Brenelli [31]	2014	59	50	BCT 59/59	52/59	7/59	56/59	NR	NR	LD 1/59, MP 1/59	34.4	3/59	1/59	III

Abbreviations: Breast-Conserving Therapy (BCT), Mastectomy (MST), Radiotherapy (RT), Chemotherapy CT), Hormonal Therapy (HT), Preoperative (prO), Post-operative (poO), Latissimus Dorsi (LD), Modified (m), Breasts (b), Prosthesis (Pr), Transverse Rectus Abdominis Myocutaneous Flap (TRAM), Ductal Intra-epithelial Neoplasia (DIN), Lobular Intra-epithelial Neoplasia (LIN), ductal (duct), lobular (lob), Not Specified (NS), Not Reported (NR), Deep Inferior Epigastric Perforator Flap (DIEP), Immediate Tissue Expander Reconstruction (ITER), Tissue Expander reconstruction (TE), nipple-sparing (ns), Mastopexy (MP).

[a] One case of contralateral breast cancer was noted.

[b] Two patients received AFG for congenital deformities (Poland syndrome and breast asymmetry).

[c] Twenty-two reconstructions were prophylactic or histological data showed no tumour.

[d] Prosthetic reconstruction was performed after the last session of AFT.

[e] There were two cases of carcinoma of the contralateral breast, one which showed simultaneous metastatic bone and liver disease.

[f] Of the 278 patients receiving mastectomies, 34 were prophylactic and data were missing for 21 patients.

[g] One patient died of disease, without specification.

[h] One additional patient had a medullary carcinoma and one Paget's disease.

[i] Three patients had phyllodes.

[j] Missing data: 11/151.

[k] A patient's bone metastasis was considered a natural progression of the disease.

Radiological follow-up

The radiological outcome after AFG was studied in a total of 17 studies [9-12,16,18,19,24,31,32,36,39,41,43-46] (Table 3/ Figure 2). Mammograms (12 studies, n= 2508) [10,11,16-19,24,31,32,41,43,44] sonograms (six studies, n= 217) [11,16,32,39,43,45] and magnetic resonance imaging (MRI) scans (eight studies, n= 424) [9,11,12,16,32,36,43,44] were used. Cancer recurrence was observed in one patient on mammogram and MRI scan [32]. A pooled analysis of the results showed the following radiological findings:

- Oil cysts were described based on mammograms in 14.3% of cases (95% CI 12.2-16.4), sonograms in 26.7% (95% CI 20.8-32.6) and MRI scans in 21.4% (95% CI 17.1-25.7).
- Fat necrosis on mammograms was seen in 9.0% of cases (95% CI 4.2-13.9), sonograms in 11.3% (95% CI 6.6-16.0) and MRI scans in 7.3% (95% CI 4.0-10.3).
- Macro- and micro-calcifications were most frequently seen on mammograms and were described in 8.7% (95% CI 3.3-14.5) and 5.4% (95% CI 2.3-8.5), respectively, versus 1.3% (95% CI 0.1-4.9) and 1.3% (95% CI 0.3-4.1) on sonograms.
- Mammograms showed irregular lumps in 5.1% (95% CI 1.3-15.1) and benign new nodules in 2.3% (95% CI 1.3-3.4).
- Suspicious images that required biopsies were seen in 3.7% (95% CI 0.1-7.2) of post-operative mammograms, 3.7% (95% CI 0.4-13.9) of post-operative sonograms and 3.3% (95% CI 0.0-19.5) of post-operative MRI scans. All biopsies were negative for cancer recurrence.
- Cancer recurrence was seen in 4.2% (95% CI 0.0-23.8) on mammogram and sonogram.

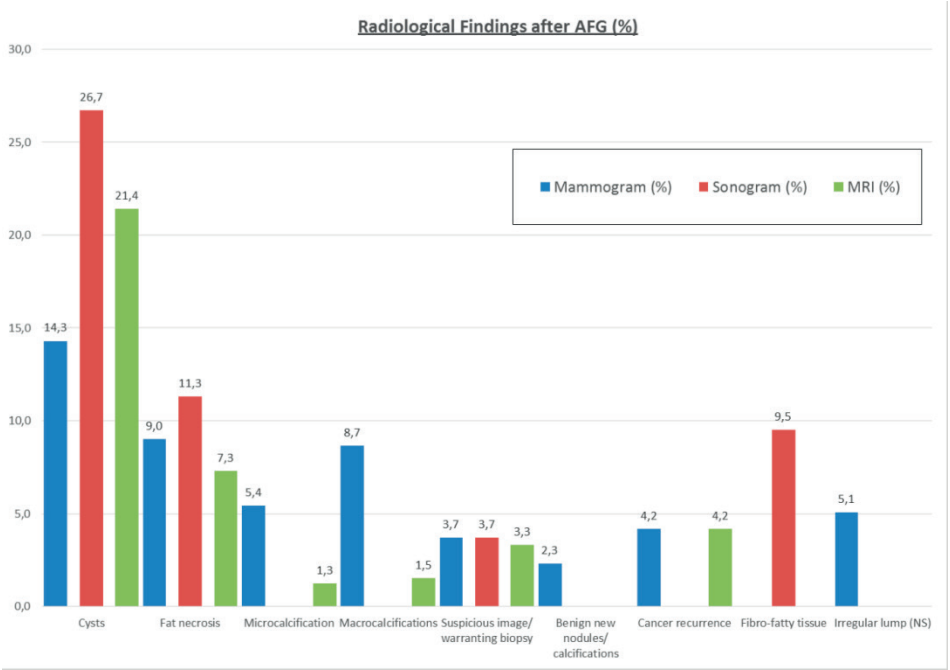
Table 3: Radiological follow-up: Overview of physiological-/ mammographic-/ sonographic- and/ or MRI findings after AFG

Study	Year	Pat. # (breasts)	Timing of imaging	Follow up, months (mean/ median/ actual)	Physical Findings	Mammographic findings	Ultrasound findings	MRI findings	BI-RAD
Pierrefeu-Lagrange [16]	2006	30	1 y postop	12 (actual)	NR	Oil Cysts: 6/36, Fatnecrosis: 1/36, Microcalcifications: 4/36, Suspicious image: 1/36	Normal: 20/34, Oil Cysts: 12/34, Fatnecrosis: 2/34, Microcalcifications: 0/34, Suspicious image: 1/34	Normal: 24/30, Oil Cysts: 7/34, Fatnecrosis: 2/34, Micro-calcifications: 0/34, Suspicious image: 1/34	NR
Missana [44]	2007	69 (74)	postop NS	12 (11.7) (mean)	NR	Fatnecrosis: 5/74, Microcalcifications: 0/74, Suspicious lesions: 0/74	NR	Fat necrosis: 5/74	NR
Gosset [11]	2008	21	Sx: Preop + > 1 y postop Mx: 1 y postop	31.2 (mean)	NR	Microcalcifications: 4/21, Macrocalcifications: 3/21, Oily cysts: 5/21, Radiologic opacity + calcifications: 1/21	Oil cysts: 12/21, Fatnecrosis: 4/21, Fibro-fatty tissue: 2/21	Oil cysts: 6/19, Fatnecrosis: 3/19	NR
Delay [46]	2009	880	postop NS	Up to 120	NR	Oil Cysts: 132/880	NR	NR	NR
Illouz [10]	2009	670 (820)	6 m,1 y postop	136 (mean)	NR	BIRADS only	NR	NR	NR
Rietjens [18]	2011	158	NS	18.3 (mean)	NR	Benign images NS: 5/77	NR	NR	II: 17/20, III: 3/20, Of which 2/3 were reclassified as II 6 months later and one 12 months later.
Petit [19]	2011	513	Preop +19.2 m postop (r 1-107 m) From 119/143 pts from the BCT group only	19.2 (mean)	13/646 Lipo-necrosis	Benign calcifications: 7/119, Benign opacity masses: 2/119, Suspicious lesions: 3/119, Biopsy showed: Fatnecrosis: 2/7, Scarring: 2/7, Fibrocystic lesions: 1/7, Local breast relapses: 2/7	NR	NR	NR

Study	Year	Pat. # (breasts)	Timing of imaging	Follow up, months (mean/ median/ actual)	Physical Findings	Mammographic findings	Ultrasound findings	MRI findings	BI-RAD
Beck [41]	2011	10	Preop + 3 y postop	Up to 36	2/10 nodules (oily cyst)	Oil Cyst: 1/10, Fatnecrosis: 5/10	NR	NR	NR
Perez-Cano[9]	2012	67 (68)	Preop + 6, 12m postup	12 (actual)	10/67 injection cysts	NR	NR	Small (<2cc) benign cysts 46/67	NR
Gentile [12]	2013	100	Preop + 6, 12 m postop	12 (actual)	NR	NR	NR	Cystic formation + Macrocalcification: 1/33	0: 30/670, I: 315/670, II: 208/670, III: 117/670
Constantini[32]	2013	24	Preop + 1 y postop	12 (actual)	NR	New calcification: 0/24, New simple Cysts: 0/24, New oil Cyst: 0/24, Fatnecrosis: 1/24, Benign nodules: 2/24, Cancer recurrence: 1/24	Micro-/macrocalcification:0/24, New simple cyst: 0/24, Oil Cyst: 23/24, Fatnecrosis: 4/24, New nodules: 0/24, Suspicious image: 1/24	New calcification: 0/24, New simple Cysts: 0/24, New oil Cyst: 16/24, Fatnecrosis: 8/24, New benign nodules: 0/24, Cancer recurrence: 1/24	I: 7/20, II: 13/20, III: 0/20
Ihrai [24]	2013	64	12 m postop	46.44 (mean)	NR	Benign scar tissue: 1/64	NR	NR	NR
Derder [43]	2014	10	Postop 12 m	68	NR	Oily cysts 1/10	Oil cysts: 1/10	Oily cysts 1/10	NR
Kim [39]	2014	102	Preop/ postop NS	28.7 (mean)	3 Nodules (biopsy proven fatnecrosis)	NR	Fat necrosis: 10/102, Oil cysts: 8/102	NR	NR
Mestak [45]	2014	30	Postop 12-30 m	21 mean	NR	NR	Cysts: 3/30	NR	NR
Li [36]	2014	105	18 m postop	18 (mean)	Small nodules: 3/105	NR	NR	- New oil cysts: 0/105 - Fatnecrosis: 0/105 - Breast masses: 0/105 - Benign-appearing calcifications: 2/105	NR
Brenelli [31]	2014	59	Postop 6 m + annually	34.4 (mean)	NR	Oil cysts: 3/59, Irregular lump 3/59, Macrocalcification 6/59, Microcalcification 3 /59	NR	NR	NR

Abbreviations: Not Reported (NR), Year (y), Month (m), Not Specified (NS), Sonogram (Sx), Mammogram (Mx), BI-RAD (Breast Imaging-Reporting and Data System), Range (r), patients (pts), Magnetic Resonance Imaging (MRI), Post-operative (postop), Preoperative (preop).

Figure 2: Radiological Findings after AFG (mammogram, sonogram, MRI).



Complications

In a total of 33 studies, 461 complications were identified in 5502 patients (Table 4/ Figure 3) [8-12,14,17-22,24,25,27,30-37,39,41-45,48-52]. The average values for complications were calculated by pooled analyses:

- The total reported complication rate was 8.4% (95% CI 7.6-9.1).
- New palpable nodules were seen in 11.5% of cases (95% CI 9.0-13.9).
- The formation of cysts was reported in 6.9% (95% CI 4.5-9.3) of cases, followed by hematoma in 6.3% (95% CI 5.1-7.5) and fat necrosis in 4.0% (95% CI 3.4-4.6).
- Other reported complications included calcifications in 5.2% (95% CI 3.1-7.3), striae of the breast in 4.4% (95% CI 3.0-5.8), granuloma in 3.6% (0.0-19.47), infection or cellulitis of the injection site in 0.8% (95% CI 0.6-1.1), seroma in 0.8% (95% CI 0.1-1.6), donor-site infection in 0.7% (95% CI 0.04-2.8), abscess in 0.6% (95% CI 0.0-3.9), pneumothorax in 0.2% (95% CI 0.0-0.4) and delayed wound healing in 0.1% (95% CI 0.0-0.64).

In one study, an infected haematoma was punctured and the patient was treated with antibiotics [22]. Infection or cellulitis of the injection site was treated with

antibiotics, drainage and/or ice packing in 43 patients. A more serious complication - pneumothorax - was reported in four patients [17,19,33,53], and pleural drainage was used in three. Fat necrosis and cysts were surgically removed or aspirated, and histological or cytological analysis of the biopsied material confirmed the diagnosis in all cases.

Table 4: Complications: Overview of complications and management

Study	Year	Pat. #	Complications	Management
Missana [44]	2007	69	Hematoma: 0/69, Infection: 0/69, Cellulitis: 0/69, Fat emboli: 0/69	
Delay [8]	2008	42	Infection: 2/42	Antibiotics, analgesics, antipyretics, Ice, stitch removal
Gosset [11]	2008	21	Infection: 2/21	Antibiotics, analgesics, antipyretics, Ice, stitch removal
Delay [17]	2009	880	Infections: 6/880 Intraoperative pneumothorax: 1/880 Fat embolism: 0/880, Fat necrosis: 26/880	Drainage, topical treatment, antibiotics, ice Pleural drain placement
Illouz [10]	2009	820	Striae: 36/820, Hematoma: 88/820 Infection: 5/820	Antibiotics
Sinna [33]	2010	200	Infection: 2/200 Pneumothorax: 1/200 Palpable nodule: 5/200 (cytosteatonecrosis)	Antibiotics Pleural drainage Surgical removal
Rietjens [18]	2011	158	Liponecrosis: 5/191 Abces: 1/191 Cellulitis: 1/191, Donor site complications: 0/191	Drained Oral antibiotics
Petit [19]	2011	513	Fat necrosis: 13/646, Infection: 3/646, Seroma: 1/646, Pneumothorax: 1/646	
de Blacam [48]	2011	49	Fat necrosis: 4/111 Oil Cyst: 2/111 Infection: 1/111 Hematoma's and implant ruptures: 0/111	Ultrasonic liposuction of fat necrosis: 2/4 Surgical removal: 1/2 Antibiotics
Sarfati [37]	2011	28	Complications, donor/ recipient site (NS): 0/28	
Beck [41]	2011	10	Infection: 1/10 Complications (NS): 9/10	Antibiotics only
Perez-Cano[9]	2012	67	Donor site subcutaneous hematoma 1/67 Injection site cysts 10/67	Conservative management Conservative management
Salgarello [49]	2012	16	Seroma: 0/16, Hematoma: 0/16, Infection: 0/16, Capsule contracture grade 1:16	
Seth [20]	2012	69	Fat necrosis: 1/99	Conservative management
Doren [25]	2012	278	Palpable mass: 64/278 Fat necrosis: 45/278	Ultrasound and/ or tissue diagnosis
Gentile [12]	2013	100	Hematoma (Coleman group): 1/33	

Study	Year	Pat. #	Complications	Management
Delay [42]	2013	31	Infection: 0/31, Haemorrhage: 0/31, Pneumothorax: 0/31, Fatty embolism: 0/31	
Ho Quoc [51]	2013	1000	Infections: 8/1000, Delayed wound healing: (NS): 1/1000, Fat Necrosis: 31/1000	
Costantini [32]	2013	24	Procedure related complications (NS): 0/24	
Sarfati [27]	2013	68	Seroma: 4/68 Complications (NS): 0/68	Resolved spontaneously: 3/4 Prosthesis explantation: 1/4
Ihrai [24]	2013	64	Infection of harvesting area: 1/64	Antibiotics only
Hoppe [22]	2013	28	Infection: 1/28, Liponecrosis 4/28, Granuloma: 1/28 Haematoma donor site: 1/28	Haematoma: puncture and antibiotics
Thekkinkattil [50]	2013	10	Painful lump: 1/10 Fat necrosis: 1/10, Deformities: 0/10, Pain: 0/10 Infection: 0/10, Dysesthesia 0/10	Fat necrosis on sonogram
Derder [43]	2014	10	Infections: 0/10	/
Kim [39]	2014	102	Fat necrosis: 10/102, Oil cysts: 8/102 Palpable mass: 3/102	Biopsy (fat necrosis)
Hitier [21]	2014	150	Infection: 0/150, Hematoma: 0/150, Pneumothorax: 0/150, Embolism: 0/150 Persistent pain: 0/150 Clinically palpable nodules of fat necrosis: 3/150	Biopsied (all fat necrosis)
Semprini [30]	2014	151	Ecchymoses (harv. site): 3/151	/
Molto-Garcia [14]	2014	37	Positive margins in the lumpectomy bed: 2/37 Hematoma/bleeding: 1/37, Oily cysts: 6/37, Calcifications: 5/37, Fat necrosis: 4/37	Re-excision
Mestak [45]	2014	30	Infection: 1/30 (PureGraft group) Solitary cysts: 3/30 (2/30 Puregraft, 1/30 Centrifugation)	Antibiotics
Brenelli [31]	2014	59	Fat necrosis: 2/59, Cellulite 1/59	
Chiu [34]	2014	282	Fat necrosis: 3/282 (2 in group A, one in group B) Infection: 2/282 (1 in both groups) Induration and/ or calcification: 15/282 (10 in group A, 5 in group B)	
Li [36]	2014	105	New oil cysts: 0/105, Fat necrosis: 0/105, Breast masses: 0/105, Benign-appearing calcifications: 2/105 Small nodules: 3/105	Aspiration (revealing Fat necrosis)
Ho-Quoc [35]	2014	31	Complications (NS): 0/31, Oil cysts NR	

Abbreviations: Not specified (NS), Not reported (NR).

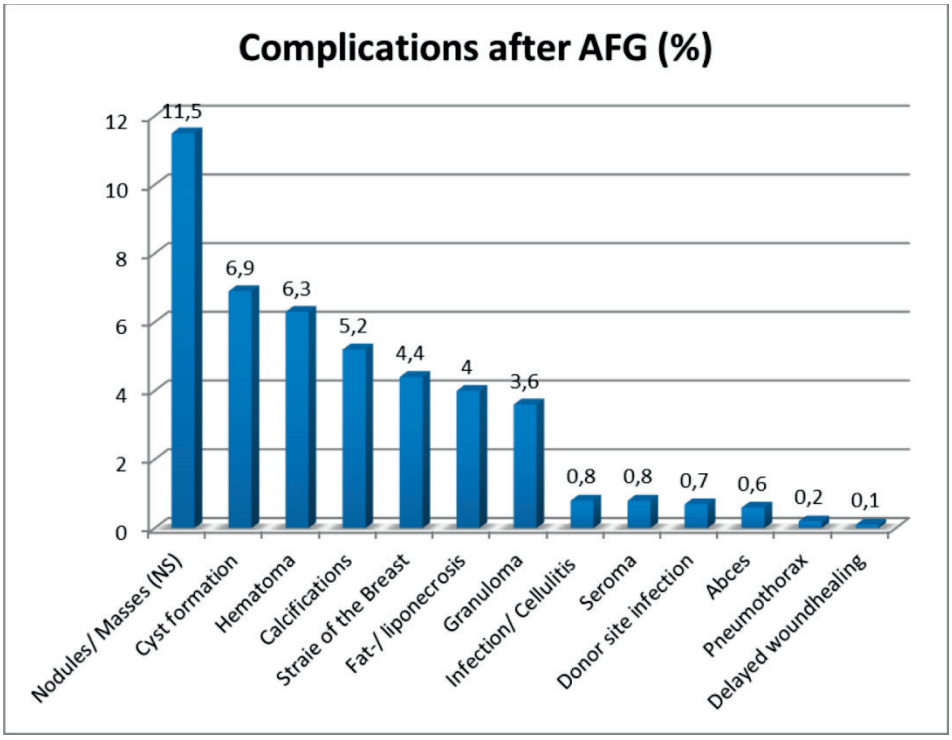


Figure 3: Complications after AFG

Fat grafting technique

All 42 studies described the methods of preparation and grafting of the adipose tissue (Table 5). General anesthesia alone was reported in 16 studies [8,12,14,15,17,21,35,36,42-45,47,48,51]. In 26 studies [8-10,12,17,18,20-24,27,31,33,36,37,39,41,43-50], the abdomen was the primary donor site; in the case of insufficient fat or odd fat distribution, fat was taken from the gluteal area or the arms. Infiltration was preferably carried out with Coleman's solution or Klein's solution. The most frequently reported harvesting method was manual aspiration followed by centrifugation ranging from 1000 to 3500 rpm for periods ranging from 20 seconds to 5 minutes, as the primary form of preparation. For injection, blunt cannulas were used most often, with diameters ranging from 1.0 to 3.0 mm or 14-18 gauge. Twenty-seven studies described a multiplane injection technique with a retrograde form of injection (on withdrawal), complementing autologous breast reconstruction techniques [8-10,12,14,15,17,18,20-23,27,32-34,36-39,43-45,48-51]. A subcutaneous or subglandular approach (as described in the Lipomodelling Guidelines as published by British Association of Plastic, Reconstructive and Aesthetic Surgeons

(BAPRAS) and British Association of Surgical Oncology (BASO)[54] was used most often in breast conservative surgical techniques. Some authors reported performing rigottomies to resolve fibrous adhesions [9,18,30,35,38,42,44,45,48,49,51] or overcorrection to compensate for post-operative resorption [8,21,38,39,43,46,48]. In 338 patients, one session was sufficient with a mean injected volume of 68.5 cm3, 238 patients had two sessions (mean injected volume of 142.9 cc) and 43 patients had three sessions (mean injected volume of 76.0 cc). Four or five sessions were needed in two cohorts of four patients (mean injected volume of 145 and 165 cc, respectively), and five patients were eventually treated six times with 140 cc being injected. No significant association was found between the volume of the initial fat graft and the number of sessions or between the form of reconstruction and the number of sessions.

Table 5: Fat grafting technique: Overview of the form of anaesthesia, donor site, infiltration solution, harvesting-, preparation-, and injection technique used. Addition of supplementation, rigotomies or overcorrection, the number of sessions and the injected volume are subsequently given.

Reference	Year	# pat.	Anesth.	Donor Site	Infiltration	Harvesting	Preparation	Supp	Inject. technique	Rig.	Over Corr. (%)	No. sessions	Volume injected/ breast(cc)/ session(mean)
Pierrefeu-Lagrange [16]	2006	30	NR	NR	NR	NR	NR	NR	NR	NR	NR	5 (2), 25 (1)	34-290 (164.7)
Missana [44]	2007	69	Gen	Abd, Hip, Th, GM, back	No infiltration	Manual	C/ 3500 rpm for 4 min.	NR	17/18 G cannulas, multiplane retrograde manner	Yes	No	2 (3), 9 (2), 63 (1)	18-360
Delay [8]	2008	42	Gen	Abd, Tr, Kn, Th	Ropivacaine	Manual	C/ 3000rpm for 3 min.	NR	1,5 mm cannulas, multiplane retrograde manner	NR	40	1 (3), 9 (2), 32 (1)	43-302
Panettiere [46]	2009	61	NR	Abd, Hip, Tr	NaCl/Lido/ Epi	Manual	NaCl washing	NR	14 G needle, localized (at defects) injection	NR	15	1-7	8-50 (24.5)
Delay [17]	2009	880	Gen	Abd, Th, KN, Tr	NR	Manual	C/ 3200 rpm for 3 min.	NR	Multiplane (deep-superficial) retrograde manner	NR	NR	NR	NR
Illouz [10]	2009	820	NR	Abd. Fl, Th, KN	NaCl/ Epi	Manual	Decanting: 10-15 min.	NR	2,5 mm cannula, multiplane (subcut. and intraglan.) retrograde manner	NR	NR	1-5	25-180 (145)
Sinna [33]	2010	200	Gen: Loc 175: 25	Abd, GM, Tr	NR	Manual	C/ 3200 rpm for 3 min.	NR	Multiplane (subcut., pect., LD flap) retrograde crossing an-shaped manner	NR	NR	7 (3), 37 (2), 156 (1)	35-405 (176
Serra-Renom [47]	2010	65	Gen	Abd.	NR	Manual	C/ 3000 rpm for 5 min.	NR	1. Expander placement + upper quadrant (subcut, intramusc.) injection 2. > 3 months, prosthesis switch + lower quadrant (subcut./ intramusc.) injection 3. NAC reconstr. + localized injection	NR	NR	1 (4), 5 (3), 59 (2)	75-150
Rigotti [29]	2010	137	Loc+DS	KN, Abd, Tr	cNaCl/ Lido/ Epi	CT	C/ 3000 rpm	NR	NR	NR	NR	NR	NR
Rietjens [18]	2011	158	Gen:19% Loc:81%	Abd, Th, Hip, KN	KS	Manual	C/ 3000 rpm for 3 min.	NR	Blunt Colemans cannula, multiplane, retrograde manner	Yes	NR	1 (5), 25 (2-4), 132 (1)	6-183 (83.5)

Reference	Year	# pat.	Anesth.	Donor Site	Infiltration	Harvesting	Preparation	Supp	Inject. technique	Rig.	Over Corr. (%)	No. sessions	Volume injected/ breast(cc)/ session(mean)
Petit [19]	2011	513	Gen or Loc (NS)	NR	KS	Manual	C/ 3000 rpm for 3 min	NR	1-cc LLS injection (NS)	NR	NR	1-6	5-400 (107.3)
de Blacam [48]	2011	49	Gen	Abd, Fl, Hip, Th	LRS/ Lido/ Epi	Manual	C/ 3000 rpm for 3 min.	NR	Colemans cannula, multiplane injection	Yes	10-20%	1 (4), 6 (3), 28 (2), 33 (1)	11- 460 (107)
Sarfati [37]	2011	28	NR	Abd, KN, Fl, GM	NR	MA	C/ 3000 rpm for 3 min.	NR	Multiplane (subcut, muscular, retromuscular) manner.	NR	NR	4 (3), 15 (2), 9 (1)	70-275 (115)
Beck [41]	2011	10	Gen:Loc 2:8	Abd/ Hip	CS	CT	Coleman technique	NR	Coleman technique	NR	NR	1	(67.5)
Petit [28]	2012	321	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Perez-Cano[9]	2012	67	Gen 97% Loc 3%	Abd	NR	NR	ADRC-enriched fat grafts	ADRC-enriched fat grafts	Multidirectional manner using microdroplet dispersion syringes	Yes	NR	24 (2), 43 (1)	140 (35-250)
Salgarello [49]	2012	16	NR	Abd, Fl, Tr, Th, KN	NaCl/ Epi/ Xylocaine	Manual	C/ 3000 rpm for 3 min.	NR	17 G blunt Coleman cannulas, multiplane manner	Yes	NR	7 (3), 9 (2)	MST 60-150 (102.8) BCT 60-130 (88.6)
Seth [20]	2012	69	Loc:32% Gen:68%	Abd, Hip, Fl	CS	CT	Sedimentation	NR	Multiplane (subcut., intramusc.) manner	NR	NR	1-4	20-50
Doren [25]	2012	278	NR	Th, Abd, Fl	NaCl/ Lido/ Epi	MA	Sedimentation	NR	Localized subdermal injections	NR	NR	8 (3), 70 (2), 200 (1)	5-200 (50)
Petit [26]	2013	59	NR	NR	NR	CT	Coleman technique	NR	Coleman technique	NR	NR	1	NR
Gentile [12]	2013	100	Gen	Abd	NR	Manual	C/ 3000 rpm for 3 min	PRP vs AFG	1-2 mm cannulas, multiplane, retrograde manner	NR	NR	NR	(120.0)
Delay [42]	2013	31	Gen	NR	NaCl/ Epi	Manual	C/ 3000 rpm for 40 sec or 3 min	NR	Doubleplane, localized injection	Yes	NR	17 (2), 14 (1)	90-316
Ho Quoc [51]	2013	1000	Gen	NR	NaCl/ Epi	Manual	C/ 3000 rpm for 20 sec or 3 min	NR	2 mm cannula, multiplane (subpect., pect., subgland., gland., subcut.) retrograde manner	Yes	NR	1-3	NR

Reference	Year	# pat.	Anesth.	Donor Site	Infiltration	Harvesting	Preparation	Supp	Inject. technique	Rig.	Over Corr. (%)	No. sessions	Volume injected/ breast(cc)/ session(mean)
Costantini [32]	2013	24	NR	NR	NaCl/ Epi	CT	C/ 3000 rpm for 3 min.	NR	17 G blunt cannula, multiplane manner	NR	NR	3 (4), 5 (3), 9 (2), 7 (1)	(114.8)
Sarfati [27]	2013	68	Gen	Abd, Fl, Tr, Th	NR	MA	C/ (NS)	NR	Multiplane (subcut., retro-/ intramusc.) retrograde, manner	NR	NR	1-6	70-275 (115)
Riggio [23]	2013	60	Loc	Abd, Fl, KN, Th	NaCl/ Epi/ Lido or Mepivacaine	Manual	Decanting (NS)	NR	17 G cannula multiplane, retrograde manner	NR	NR	1 (4), 5 (3), 17 (2), 37 (1)	(46.84)
Ihrai [24]	2013	64	NR	Abd	CS	Coleman	Coleman technique	Coleman technique	Coleman technique	NR	NR	1 (5), 2 (4), 5 (3), 16 (2), 40 (1)	10-80 (38)
Hoppe [22]	2013	28	Gen:47% Sed:53%	Abd, Wst, LR, Arm	NaCl/ Lido/ Epi/ NaBic	BEAULI	BEAULI + Decanting: 5-10 min	NR	2 mm cannulas, multiplane (subcut., intrapect.) manner	NR	NR	2-10	(1020) in total
Thekkinkattil [50]	2013	10	NR	Abd, Fl	NaCl/ Epi/ Chirocaine	Manual	C/ 3000 rpm for 3 min	NR	Multiplane (LD flap) injection	NR	NR	3 (4), 4 (3), 3 (2)	60-768 (529)
Fiaschetti [15]	2013	10	Gen	NR	KS	NR	C/ 3000 rpm for 4 min	NR	Coleman microcannula, multiplane retrograde manner	NR	NR	NR	NR
Derder [43]	2014	10	Gen	Abd, Tr, Th, KN, LR	NaCl/ Epi	vMA	C/ 3000 rpm for 3 min	NR	2 mm cannula, multiplane injection	NR	Slight	1 (3), 5 (2), 4 (1)	200-500 (285)
Del Vecchio [40]	2014	30	NR	Th	NaCl/ Epi	MA	C/ 30-40 g for 3 min	BRAVA: 3 weeks preoperative (NS)	14 G Coleman needle, single plane (subcut.) manner	NR	NR	1	(606.7)
Longo [38]	2014	21	Loc+sed	NR	NR	Manual	C/ 3000 rpm for 3 min	NR	Blunt Coleman cannula, multiplane (subcut., submusc.) retrograde manner	Yes	30%	5 (6), 4 (5), 1 (4), 11 (3)	330-720 (506.68)

Reference	Year	# pat.	Anesth.	Donor Site	Infiltration	Harvesting	Preparation	Supp	Inject. technique	Rig.	Over Corr. (%)	No. sessions	Volume injected/ breast(cc)/ session(mean)
Kim [39]	2014	102	NR	Abd, Fl, Th	NaCl/ Lido/ Epi	Manual	C/ 3000 rpm for 3 min.	NR	Coleman cannula, multiplane manner	NR	Yes (NS)	4 (3), 25 (2), 73 (1)	10-183
Hitier [21]	2014	150	Gen	Abd, GM	NaCl/ Epi	Manual	C/ 3000 rpm for 20 sec	NR	2 mm cannula, multiplane, retrograde manner	NR	140% (aim)	1 (3), 9 (2), 140 (1)	20-272 (94)
Semprini [30]	2014	151	NR	Tr, Fl, Abd	NaCl/ Epi/ mepivacaine	Manual	C/ 1000 rpm for 1 min	NR	Microcannula injection (NS)	Yes	NR	20 (3), 46 (2), 85 (1)	20-272
Molto-Garcia [14]	2014	37	Gen	NR	CS	CT	Decanting 10-15 min + Washing (NS)	NR	Localized, multiplane manner	NR	NR	NR	(65)
Mestak [45]	2014	30	Gen	Abd, Fl, Th	NaCl/ Epi	Manual	C/ 3000 rpm for 3 min LRS washing x2	NR	Coleman cannula, multiplane, retrograde manner	Yes	NR	NR	C: 162 PG:232
Brenelli [31]	2014	59	Loc:77.3% Gen:22.7%	Abd, Hip, KN	CS	CT	Coleman technique	NR	Coleman technique	NR	NR	2 (3), 3 (2), 54 (1)	(52.3-137.9)
Chiu [34]	2014	282	Gen + Loc (NS)	NR	LRS/ Lido/ Epi	MA	C/ 800 g for 4 min	SVF	Multiplane (subcut., intramusc., retromusc., and premusc.) manner	NR	NR	NR	(247.5)
Li [36]	2014	105	Gen	Abd, Fl, Tr, Th, KN, arm	NaCl/ Lido/ Epi	MA	NaCl washing 3-4x + cotton pad decanting	NR	14 G blunt cannula, multiplane (retro-/ intraglan., subcut.) manner	NR	NR	5 (3), 20 (2), 80 (1)	(205)
Ho-Quoc [35]	2014	31	Gen	Th, Hip	NaCl/ Epi	Manual	C/ 3000 rpm for 3 min or 20 sec	NR	2 mm cannula	Yes	NR	2	(760)

Abbreviations: Thighs (Th), Flanks (Fl), Not Specified (NS), Not Reported (NR), General Anaesthesia (Gen), Local Anaesthesia (Loc), Analgesic Sedation (Sed), Gluteus region (GM), Abdomen (Abd), Trochanteric region (Tr), Knees (KN), Lumbar Region (LR), Waist (Wst), NippleAreola Complex (NAC), Deep Sedation (DS), Lactate Ringer Solution (LRS), Stromal Vascular Fraction (SVF), Luer-Lock Syringe (LLS), Epinephrine (epi),Klein's Solution (KS), Coleman's Solution (CS), Centrifugation (C), Puregraft (PG), Range per minute (rpm), Subpectoral (subpect.), Pectoral (pect.), Subcutaneous (subcut.), Subglandular (subgl.), Glandular (gland.), Intraglandular (intraglan.), Intramuscular (intramusc.), Retromuscular (retromusc.), Preoperative (preop), Latissimus Dorsi (LD), Sodium Chloride (NaCl), Sodium Bicarbonate (NaBic), Lidocaine (lido), Gauge (G), G-force (g), Vacuum machine assisted (vMA), Machine Assisted (MA), Coleman Technique (CT).

Volume retention

Volumetric analysis was performed in two studies [15,41], with a total of 20 patients who underwent breast reconstruction supplemented with AFG (Table 6). One study used MRI and one study made use of an advanced three-dimensional (3D) measuring system in combination with computed tomographic (CT) analysis. The mean injected volume and the percentage of volume gain relative to the injected volume after a minimal period of 1 year post-operatively were 128.1 ml and 63.7%, respectively. To study the effect of (adjuvant) radiotherapy on volume retention, we combined these results with the volumetric outcomes of six studies [40,53,55-58] that reported on volume retention after AFG for indications other than onco-plastic surgery. A mean volume retention of 76.8% (range 44.7-82.6%) was seen in 681 patients who received AFG for various indications with a mean follow-up of 20 months (range 12-120) compared to 63.8% (range, 56.0%-71.5%) in 16 patients [15,41] after radiotherapy with a mean follow-up of 24 months (range 12-36).

Table 6: Volume Retention: Overview of the auxiliary method, the method of measuring volume retention, the indications and number of sessions as well as the mean total injected volume, the volume gain and the percentage of gain relative to the injected volume.

AFG after reconstruction											
Study	Year	Auxiliary method	Meth. of measuring	Indication	Pat. #/ Indication	RT	Mean preoperative breast volume (ml)	Sessions	Mean total injection volume/breast (ml)	Mean volume gain/ breast (ml)	Volume gain relative to graft volume (%)	Follow-up (months)
Fiaschetti [15]	2013	NR	MRI	*Cosmetic* *Congenital* *I-Fc* Reconstruction	*2* *2* *1* 10	 8	642.15	2	188.46	134.74	71.5	12
Beck et [41]	2011	NR	CT/ 3D	Reconstruction	10	8	NR	1	67.5	NR	55.99	36
AFG for other indications												
Study	Year	Auxiliary method	Meth. of measuring	Indication	Pat. #/ Indication	RT	Mean preoperative breast volume (ml)	Sessions	Mean total injection volume/breast (ml)	Mean volume gain/ breast (ml)	Volume gain relative to graft volume (%)	Follow-up (months)
Khouri [53]	2014	BRAVA	MRI	Cosmetic Congenital Iatrogenic I-Fc	294 45 43 94		319 210 280 290	8 (3), 73 (2), 395 (1)	367 300 350 300	293 240 280 190	79.8 79 79 64	42
Spear [58]	2014	NR	MRI/ 3D	Cosmetic	10		75.3 (r 48.6–125.5)	1	236 (r 90-324)	105.5 (r 63.8-186.8)	44.7	12
Del Vecchio [40]	2014	BRAVA	3D	Cosmetic Revision surgery	24 6		196.6	1	610.0	305.8	50.1	12
Auclair [55]	2013	Implants	3D	Cosmetic	20		164.4	NR	368	204.9	56.1	12
Khouri [57]	2012	BRAVA	MRI	Cosmetic	71		371	1	282	233 (r 60-619)	82.6	12
Fulton [56]	2003	PRP	VM	Cosmetic	65		NR	1	200-300	NR	73 (r 20-140)	-120

Abbreviations: Breast Enhancement and Shaping System(BRAVA), Magnetic Resonance Imaging (MRI), Implant-to-Fat conversion (I-Fc), Not Reported (NR), Three-dimensional (3D), Range (r), Platelet-Rich Plasma (PRP), Computer tomography (CT), Volumetric (VM)

Patient/surgeon satisfaction

In 14 studies with a minimal follow-up of 1 year after AFG treatment, patient and/or surgeon satisfaction was reported on a three- or five-point Likert scale (Figure 4). To enable comparison, all of these studies were converted to a three-point-Likert scale (Table 7). Patient satisfaction (n= 1089) [8,11,14,21,22,25,33,34,42,43] and surgeon satisfaction were each reported in 11 studies (n= 954) [8,11,21,22,33-36,42,44,49]. Patients were satisfied with the result in 93.4% of cases, and 90.1% of surgical teams reported a good result based on post-operative photographs or clinical assessment.

Table 7: Patient/ Surgeon satisfaction

				Patients satisfaction	Surgeons Satisfaction	
Study	Year	No. of patients	Follow up: months		Measurement:	
Missana [44]	2007	69	11.7 (mean)	NR	PPoPc/ two IS	Good 86.5%, Neutral 13.5%, Poor 0%
Delay [8]	2008	42	31.2 (mean)	Satisfied 90.5%, Neutral 9.5%, Dissatisfied 0%	PPoPc/ two IS	Good 90.5%, Neutral 9.5%, Poor 0%
Gosset [11]	2008	21	31.2 (mean)	Satisfied 90.5%, Neutral 9.5%, Dissatisfied 0%	PPoPc/ two IS	Good 90.5%, Neutral 9.5%, Poor 0%
Sinna [33]	2010	200	14.5 (median)	Satisfied 100%, Neutral 0%, Dissatisfied 0%	PPoCc/ two IS (NS) + one secretary (NS)	Good 100%, Neutral 0%, Poor 0%
Salgarello [49]	2012	16	15 (mean)	NR	PPoPc/ one IS + TS	Good 93.7%, Neutral 6.3%, Poor 0%
Doren [25]	2012	278	28 (median)	Satisfied 98%, Neutral 0%, Dissatisfied 2%		NR
Delay [42]	2013	31	78 (mean)	Satisfied 100%, Neutral 0%, Dissatisfied 0%	TS (NS)	Good 93.5%, Neutral 6.5%, Poor 0%
Hoppe [22]	2013	28	31.2 (mean)	Satisfied 88.9%, Neutral 3.7%, Dissatisfied 7.4%	PPoPc/ two IS	Good 67.9%, Neutral 21.4%, Poor 10.7%
Derder [43]	2014	10	68 (mean)	Satisfied 90%, Neutral 0%, Dissatisfied 10%		NR
Hitier [21]	2014	150	22.3 (mean) r 12-120)	Satisfied 86.7%, Neutral 11.3%, Dissatisfied 2%	PPoPc/ TS	Good 98.7%, Neutral 1.3%, Poor 0%
Molto-Garcia [14]	2014	37	12 (actual)	Satisfied 97%, Neutral 2%, Dissatisfied 1%		NR
Chiu [34]	2014	282	23.7/23 (mean)	Satisfied 85.5%, Neutral 0%, Dissatisfied 14.5%	PPoPc/ one IP	Good 85.8%, Neutral 0%, Poor 14.5%
Li [36]	2014	105	18 (mean)	NR	Three IS (NS)	Good 83.8%, Neutral 0%, Poor 16.2%
Ho-Quoc [35]	2014	10	72 (mean)	Satisfied 100%, Neutral 0%, Dissatisfied 0%	TS (NS)	Good 100%, Neutral 0%, Poor 0%

Abbreviations: Pre-/post-operative photo comparison (PPoPc), Pre-/post-operative clinical comparison (PPoCc), Independent Surgeon (IS), Treating Surgeon(s) (TS), Independent Physician (IP), Not Reported (NR).

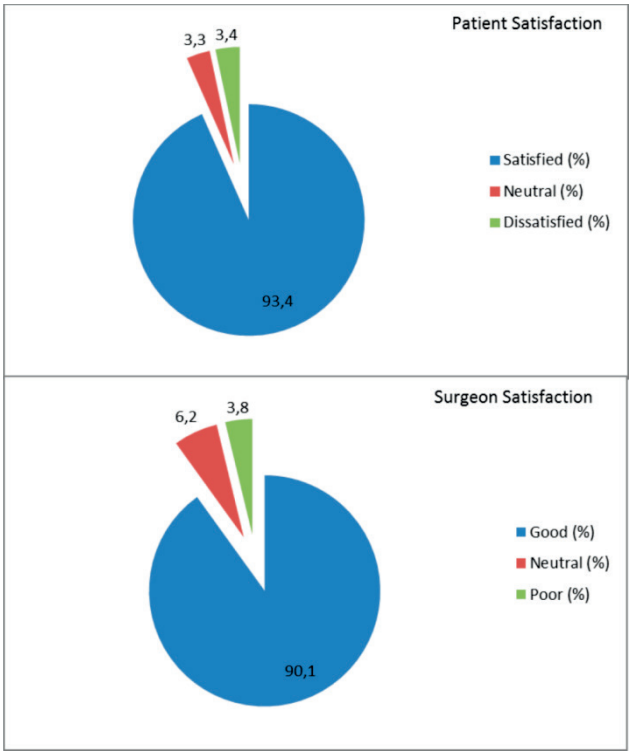


Figure 4: Patient-/ surgeon satisfaction

Risk of bias across studies

A comprehensive overview of the risk of bias across studies is given in Appendix 1.

Discussion

Summary of evidence.

Oncological safety

Based on the available evidence, breast reconstruction with the use of AFG after MST or BCT does not seem to increase the risk of cancer recurrence. The local and distant recurrence rates in this study were lower than those of patients who underwent MST with immediate breast reconstruction, as reported by Petit et al. (LRR: 2.5% vs. 5.2%; DR: 2.0% vs. 13.9%) [59]. They were also lower than the reported recurrence rates after BCT with whole-breast irradiation (LRR 2.4% and DR 8.0%) [60]. In the recent RESTORE-2 trial [71], patients were treated with ADRC

(adipose-derived regenerative cell)-enriched fat grafting after BCT [9]. In the relatively short follow-up period of 1 year, no local recurrences occurred, which is in line with our findings. When cancer did recur, the tumor was of the same histological make-up as the primary tumor. These outcomes seem to dispel the earlier apprehension that the injection of autologous fat might stimulate cancer formation or recurrence. The oncological safety of AFG is supported by a number of experimental [61] and biochemical [62] studies, and it may be further substantiated by ongoing clinical trials. However, we should remain cautious, as larger follow-up, multicenter prospective trials are still needed to support these results. This is all the more pertinent given that several experimental studies still show potential danger of interaction between adipose-derived stem cells (ADSCs) and mammary epithelial cells as well as the potential of CD34+ progenitors in white adipose tissue to promote cancer progression [28,61,63-65].

Radiological safety

Radiological safety concerns the possible interference of AFG treatment with radiological breast cancer screening. Benign irregularities are regularly seen on radiological images after reconstruction in combination with AFG. These include oil cysts, fat necrosis, micro-calcifications and macro-calcifications. Usually, these can be easily distinguished from malignancies. Distinctions can be made based on morphology, size and distribution provided the radiologist and surgeon communicate clearly with each other [66,67]. When we compare our results with those reported by Piper et al. [68], who described radiologic outcomes of patients 2 years after onco-plastic breast reconstruction, we see that more fat necrosis was observed (9.0% vs. 4.7%) and more biopsies were performed based on suspicious radiological findings (3.7% vs. 1.6%) after treatment with AFG. Nevertheless, all histological analyses of these biopsies showed a benign result (fat necrosis mostly). With the steady advances made in radiological diagnostic accuracy over time, positive identification of benign lesions may be expected to further improve in the future.

Complications

The overall combined complication rate of AFG treatment was 8.4% (95% CI 7.6-9.1). The complication rate within 12 months after AFG as well as the severity and the number of complications per patient were lower than those described after reconstructive breast procedures performed with breast implants and/or myocutaneous flaps [69].

Fat grafting technique

Current fat grafting techniques are based primarily on the surgeon's experience or expert opinions rather than on evidence-based research. Only a few studies report the effects of grafting methodology, such as harvesting location and aspiration technique, on relevant outcomes, often with inconsistent results. For instance, two studies [70,71] reported that harvest location did not affect cell survival, whereas a more recent paper [72] reported higher numbers of adipocytes and colony-forming units in trochanteric harvested fat relative to other harvest locations. With regard to aspiration, Erdim et al. showed that 6-mm suction cannulas yielded the highest number of viable adipocytes, while inadvertently producing larger fat lobules, which in turn have been demonstrated by Ohara et al. to decrease graft survival rate [73,74]. Finally, with regard to preparation and injection, the recently published systematic review by Strong et al. showed higher retention rates with centrifugation and slow injection [75]. Although various other questions have emerged with regard to the ideal technique, the findings of this study can lead to further experimental as well as clinical studies to define how and which specific aspects (aspiration, preparation, supplementation, injection, etc.) of the grafting technique contribute to the aesthetic results [70,76-81]. Until then, the authors recommend the technique best suited for the specific indication and the previous form of reconstruction, which is thoroughly described in various protocols [82]. In summary, autologous (flap) reconstruction requires a multilayer injection approach with special emphasis on the flap itself for improved revascularization. Implant reconstruction can be complemented by localized intrapectoral or subcutaneous injections when dealing with implant replacement. In BCT, subglandular and subcutaneous injections are given, and infiltration of the local excision defect is performed.

Efficacy

In this review, a mean volume retention of 76.8% (range 44.7-82.6%) was seen, compared to 63.8% (range, 56.0-71.5%) in 16 patients [15,41] after receiving (adjuvant) radiotherapy. Radiotherapy did not seem to affect volume retention, but statistical analyses could not be performed due to insufficient data. Furthermore, these numbers are too small to draw conclusions on this topic. The supposition that radiation negatively affects graft take and volume retention should be the focus of future larger prospective clinical studies. High levels of both patient and surgeon satisfaction were achieved; 93.4% of patients were satisfied with their results, and 90.1% of the surgical teams scored a good result based on comparison between preoperative and post-operative photos.

Limitations

This systematic review is limited by the inclusion of almost exclusively low-level (OCEBM III/IV) cohort studies, without a control group. Furthermore, the greatest limitation lies in the fact that none of the included studies clearly differentiates important variables such as type of primary breast surgery, type of reconstruction, addition of radio-/chemotherapy and tumor staging/histology, in relation to the most important outcomes such as oncological/radiological safety and complications. Clear-cut conclusions cannot be drawn due to the same heterogeneity between studies in nomenclature used, besides several missing variables. Moreover, some studies [10,12,15,17,36,40,53] did not clearly specify the indication for AFG, which may have resulted in a reporting bias.

Conclusions

Presently, nearly all studies on the use of AFG in breast reconstruction after breast cancer are of low evidence level. Yet, these studies indicate that AFG is a promising technique, providing high satisfaction rates. Safety does not seem to be compromised as cancer recurrence rates and complication rates are not increased. Whether AFG interferes with radiological follow-up remains to be seen. Most benign irregularities can be clearly distinguished, but slightly a higher number of biopsies are taken after AFG to histologically clarify benign irregularities, although all tested negative for malignancy. In order to confirm these conclusions, prospective randomized trials with sound methodology are needed.

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Conflict of interest

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bjps.2016.03.019.

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Chapter 3

Autologous Fat Grafting in Cosmetic Breast Augmentation; A Systematic Review on Radiological safety, Complications, Volume Retention and Patient/ Surgeon Satisfaction

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Abstract

Background: Autologous fat grafting (AFG) is increasingly used in cosmetic surgery. However, its efficacy and safety are still ambiguous. Both a comprehensive overview and recapitulation of the relevant literature provide current evidence on the efficacy and outcomes of AFG in cosmetic breast surgery.

Objectives: This review provides an up-to-date overview of the literature on AFG in cosmetic breast augmentation.

Methods: A systematic review of the literature on AFG used for cosmetic breast augmentation was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. This study included selected studies that were published between January 1996 and February 2016 and reported on 10 patients or more who had a minimal mean follow-up period of 1 year.

Results: In this study, 22 articles that reported on 3565 patients with follow-up periods ranging from 12 to 136 months were included. A complication rate of 17.2% (95% CI 15.9-18.5) was seen. Indurations were the most frequent complication (33.3%, 95% CI 20.4-46.3), followed by persistent pain (25%, 95% CI 0.5-49.5), and hematoma (16.4%, 95% CI 14.5-18.4). Mammograms revealed microcalcifications (9.0%, 95% CI 6.4-11.5) and macro calcifications (7.0%, 95% CI 3.8-10.2). The mean volume retention was 62.4% (range, 44.7-82.6%), with a satisfaction rate of 92% in patients and 89% in surgeons.

Conclusions: AFG is a promising method in achieving autologous cosmetic breast augmentation with satisfactory volume retention and satisfaction rates in eight and six studies, respectively. Complications and radiological findings are comparable to those after implant augmentation. Future studies should focus on cancer occurrence and detection to further substantiate AFG safety. In addition, grafting methods and the use of auxiliary procedures to identify factors leading to better outcomes in terms of volume retention should be investigated. Finally, objective questionnaires are needed to represent patient satisfaction.

Introduction

Autologous fat grafting (AFG) is becoming an increasingly popular procedure in cosmetic surgery. With this growing popularity, the technique has been gaining acceptance for use with cosmetic breast augmentation. However, the number of questions regarding the optimal methods for fat harvesting, processing, and injecting is also increasing, as reflected by the recent paper by Longaker et al.[1]. The systematic review of Strong et al [2] recently showed higher retention rates in human studies with centrifugation, as opposed to sedimentation, and slower reinjection rates into less mobile areas. However, this same advantage could not be found in experimental animal studies and in vitro analyses. Until recently, AFG in breast augmentation was limited by the amount of fat that could be transferred to the different compartments and the increased absorption when exceeding that amount. Recent retrospective studies on larger-volume AFG in combination with compartment-expanding techniques, such as the Breast Enhancement and Shaping System (BRAVA), have shown encouraging results; [3-6] prospective trials are currently being conducted [7]. Further positive results are expected from the use of supplementation with platelet-rich plasma (PRP), which is showing improved neovascularization and long-term graft retention in experimental animal studies [8,9]. Furthermore, stromal vascular fraction (SVF) seems to increase the quantity of adipose-derived stem cells (ASCs) in the graft,[10-12] thereby promoting adipose regeneration, angiogenesis, and release of angiogenic growth factors [13,14]. In addition, the fear that the procedure interferes with cancer diagnostics and that it may promote cancer is gradually diminishing[15], which is supported by studies reporting on the safety of the technique [16-18]. As the outcomes of AFG in cosmetic breast reconstruction seem encouraging, and with its rise in popularity, it is important to assess the available evidence on the safety and efficacy of the technique. This realization is shown in the number of recently published systematic reviews on the subject with Strong et al. [2], Largo et al. [19] and Voglimacci et al. [20]. These reviews add greatly to the comprehensive overview of the current evidence on the safety, technique, efficacy, and patient-reported outcomes. The first review by Strong et al. [2] gives a thorough descriptive analysis of the articles that focus on technique-specific aspects, such as harvest site (preparation), adipose tissue isolation, and injection techniques, as well as instruments, without looking at the specific AFG indications. The second review by Largo et al. [19], while maintaining a methodology similar to the current review and reporting on comparable outcomes, includes

36 articles up to December 31 2012, covering 1453 patients. This is less than half of the current study population covered in 22 articles. The inclusion of low-level case reports/series causes a high level of heterogeneity between the studies that already report very differently on the important technical aspects that were previously mentioned. The same methodological choice regarding the inclusion of case reports/studies is found in the review by Voglimacci et al. While this report provides an update of the included articles up to July 2014, it also omits important tables on certain outcomes, such as radiological appearances and complications. The authors believe that the inclusion of such tables increases the readability. Finally, the follow-up periods of the included studies were not reported in the review by Strong et al. [2]. These follow-up periods ranged from 1 to 156 months and 6 to 156 months in the reviews of Largo et al [19] and Voglimacci et al. [20], respectively. It is known that fat retention can take 3 to 6 months before reaching a steady state [21-23], so it is essential to maintain a longer follow-up period. Therefore, our primary aim was to give an updated comprehensive overview regarding safety, technique, efficacy, and patient-/surgeon-reported outcomes of AFG for breast augmentation purposes. Our minimal sample size was 10 patients, and the mean follow-up period was at least 1 year after the last fat grafting session. By including studies up to February 20 2016, we included three new articles [24-26], one of which practiced high-volume grafting (range, 300-600 cc) and one of which added to the number of BRAVA-prepared patients. The authors believe this can add value to this paper in comparison to previous reviews. Our secondary aim was to reveal deficiencies in the current literature, which may form the basis for further research.

The research questions were as follows:

(1) In regard to women seeking cosmetic breast augmentation (P), can the use of Autologous Fat Grafting (I) provide a safe (oncological, radiological, and in regard to complications such as bleeding, infection and post-operative pain) and effective (adequate volume retention, esthetic effect, and patient satisfaction) alternative or addition (O) to other forms of breast augmentation (with implants) (C)?

(2) In regard to women seeking alternative or additional methods for cosmetic breast augmentation with the use of Autologous Fat Grafting (P), can an extensive systematic review, which includes articles up to 2016 that have a 10-patient minimal sample size and mean follow-up period of

1 year (I), reveal new deficiencies in the current literature (O) in comparison to previously published studies (C)?

Methods

This is a systematic review of the literature reporting on AFG used for augmentation of the female breast conducted according to the PRISMA statement [27]. A completed PRISMA checklist is available as Supplementary Material at www.aestheticsurgeryjournal.com. A systematic review conducted by the same authors using a similar methodology but reporting on AFG used in addition to onco-plastic breast reconstruction was recently published [28]. The PubMed, Embase.com, Wiley/Cochrane Library, and Web of Science databases were searched from inception (by JG and JCFK) up to the final screening on February 20 2016. The following terms were used (including synonyms and closely related words) as index terms or free-text words: "fat" or "adipocyte" or "lipo" and "grafting" or "filling" or "transplant." The full search strategies for all of the databases is available as Supplementary Material at www.aestheticsurgeryjournal.com. Studies that were considered potentially relevant based on the titles were stored using the RefWorks database. There was no restriction on language, type of study, or publication media. Bibliographies of the retrieved articles were manually searched for relevant and possibly missed references.

Eligibility Criteria

Original articles regarding the application of fat grafting (with or without supplementation) in cosmetic breast augmentation were found to be eligible for inclusion. In addition, all harvesting, processing, and injecting techniques were found to be eligible for this study. The articles were collected by two independent reviewers (JG and VN) and screened on the outcomes, including complications, radiological appearances, volume retention, fat grafting technique, and patient/surgeon satisfaction. Duplicate articles, case reports, or case series with a sample size <10 and articles with a mean follow-up period <12 months were excluded.

Study Selection

The abstracts of the selected studies were evaluated independently by two researchers (JG and VN). When found eligible by both reviewers, the full text article was retrieved for evaluation, data extraction, and inclusion in the systematic review. Discrepancies between the two reviewers were discussed, and if a solution was not found, a third reviewer (MM) was consulted. When a study could not be retrieved from the electronic media or the local library, the authors were contacted to request a copy.

Outcome Measures

We included the following outcomes:

(1) Complications: type and frequency of complications.

(2) Radiological safety: type and frequency of radiological appearances (warranting biopsies).

(3) Fat grafting technique, number of grafting procedures, and graft volume.

(4) Volume retention: ratio of the volume gain relative to the injected volume.

(5) Satisfaction: patient and surgeon satisfaction.

Data Collection Process

Data were extracted by one researcher (JG) using standardized tables developed for this purpose and checked by a second reviewer (VN). Data extracted from each article included authors, date of publication, number of subjects, indication for the procedure, type of study, technique used for adipocyte implantation, follow-up time, efficacy of treatment, patient satisfaction, clinical complications, volume retention and radiographic changes. Randomized controlled trials (RCTs), prospective and retrospective observational or comparative cohort studies, and case series with a sufficient sample size and follow-up were evaluated with respect to the following factors: clear description of inclusion and exclusion criteria, method of patient selection for the procedure (i.e., consecutive vs non-consecutive recruitment), adequate sample size (> 10 patients), use of objective outcomes, and sufficient duration of follow-up period. Included studies were assigned a level of evidence according to the Oxford Centre for Evidence-Based

Medicine (2011) by two independent reviewers (JG and VN). Discrepancies in scoring were discussed by all reviewers. The principal summary measures are means over follow-up periods and percentages with an actual number given between parentheses.

Statistical Analysis

The data were pooled to calculate the overall proportion with a 95% confidence interval. Due to insufficient data reported, statistical analyses of the fat grafting technique, volume retention, and patient and surgeon satisfaction could not be performed. To compare the harvesting methods in regard to volume retention, we used the Mann–Whitney U test for abnormally distributed data (Kolmogorov–Smirnov test). The Cohen's kappa coefficient was used to calculate the inter-rating observer variability of the selected articles.

Risk of Bias Across Studies

Observational studies and clinical trials without detailed randomization protocols were considered studies with a high risk of bias. A Cochrane Risk of Bias Assessment Tool: for Non-Randomized Studies of Interventions (ACROBAT-NRSI) was used for quantifying the risk of bias across the studies. A sensitivity analysis was not performed because the two studies that were considered to have a serious risk of bias [26,29] consisted of a total cohort of 24 patients.

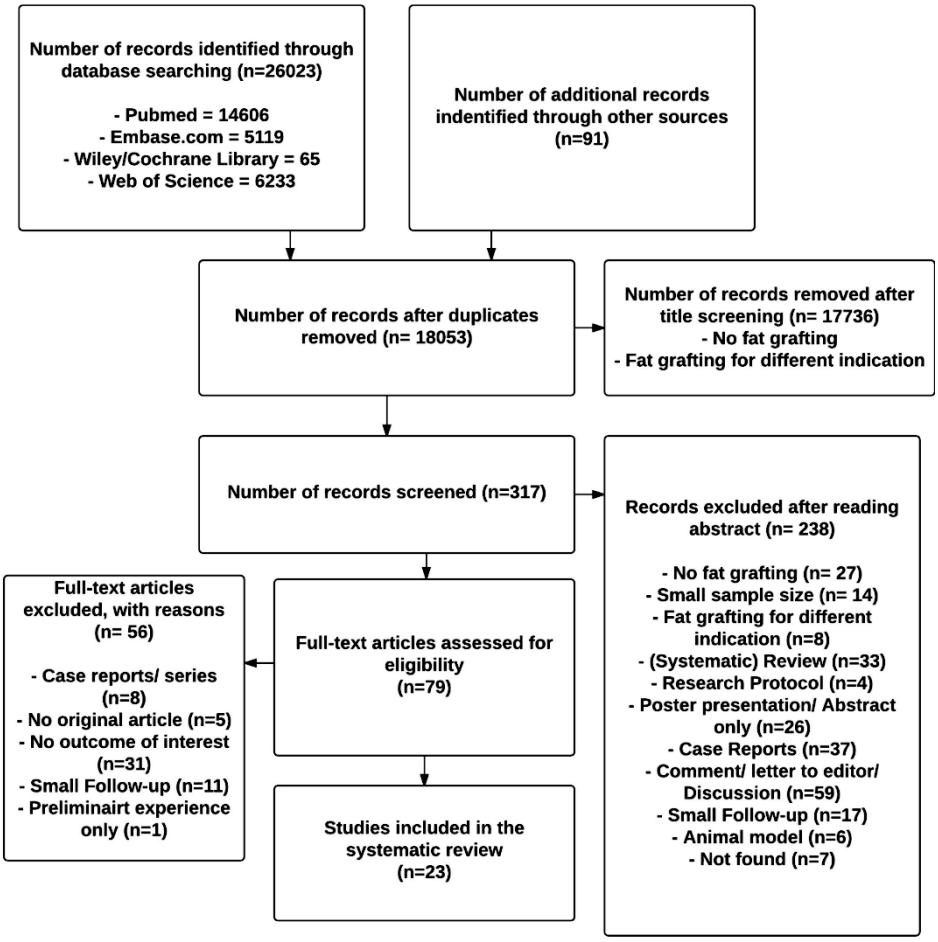

Figure 1: Flow diagram- search and selection strategy of included articles

Results

There was a high inter-rater agreement in selecting relevant articles based on the abstract screening of 0.79. There was no difference between the reviewers regarding data extraction. After screening (Figure 1), a total of 23 articles was included [4-6,10-12,21,24-26,29-41]. All of the articles were English-written articles. The risk of bias across the studies (Table 1) was measured using ACROBAT-NRSI [42] and was considered moderate in the pre-intervention (68.2% of studies), post-intervention (72.7% of studies), and overall (72.7% of studies) ratings. Two articles [12,41] described the same group of patients; therefore, one of the articles was excluded from the analyses, leaving 22 articles. Extracted data are summarized

in Tables 1-6. The included studies were published between 2003 and 2016, with 10 retrospective and 12 prospective cohort designs. There were 3 level III studies and 19 level IV studies involving a total of 3565 patients. Seven studies reported on a total cohort of patients receiving AFG for cosmetic, as well as reconstructive, purposes [6,21,33,37,39-41]. The mean follow-up period was 28.8 months (range, 12-136 months) (Table 2).

Table 1: Cochrane Risk Of Bias Assessment Tool: for Non-Randomized Studies of Interventions (ACROBAT-NRSI)

Study:	Risk of bias judgements in ROBINS-I: pre-intervention and at-intervention domains	Risk of bias judgements in ROBINS-I: post-intervention domains	Domain-level and overall risk of bias judgment in ROBINS-I
Fulton [30]	MR	MR	MR
Zheng [31]	MR	MR	MR
Hyakusoku[29]	MR	SR	SR
Zocchi [4]	MR	MR	MR
Carvajal [32]	MR	MR	MR
Delay [21]	SR	SR	MR
Illouz [33]	MR	MR	MR
Veber [34]	MR	MR	MR
Wang [35]	MR	MR	MR
Rubin [11]	LR	LR	LR
Khouri [5]	LR	MR	MR
Gentile [41]	LR	LR	LR
Fiaschetti [37]	MR	MR	MR
Auclair [38]	MR	MR	MR
Spear [36]	MR	MR	MR
Del Vecchio [39]	MR	MR	MR
Khouri [6]	MR	MR	MR
Li [40]	MR	MR	MR
Chiu [10]	LR	LR	LR
Abboud[24]	LR	LR	LR
Uda[26]	SR	MR	SR
Chiu[25]	MR	MR	MR

Abbreviations: CR, critical risk of bias (the study is too problematic to provide any useful evidence on the effects of intervention); LR, low risk of bias (the study is comparable to a well-performed randomized trial with regard to this domain); MR, moderate risk of bias (the study is sound for a non-randomized study with regard to this domain but cannot be considered comparable to a well-performed randomized trial); NI, no information on which to base a judgment about the risk of bias for this domain; SR, serious risk of bias (the study has some important problems).

Table 2: Baseline: Characteristics of included Studies

Reference	Year	Study design	Total	Cosmetic Augmentation	Reconstruction	Follow up: months (mean/ median)	Age: years (mean/ median)	Reported outcomes	Level of Evidence (OCEBM))	Fundings
Fulton [30]	2003	Retrospective cohort	65	65 (130)	-	-120	38 (mean)	VR,C,RS	IV	NFR
Zheng [31]	2008	Prospective cohort	66	66 (132)	-	37 (mean)	Range only: 19-39	E, PS, RS, C	IV	NFR
Hyakusoku[29]	2008	Retrospective cohort	12	12 (24)	-	39 (mean)	39.3 (mean)	C	IV	NFR
Zocchi [4]	2008	Retrospective cohort	181	181 (326)	-	12 (mean)	33 (mean)	VR, C, RS, E, PS	IV	NFR
Carvajal [32]	2008	Retrospective cohort	20	20 (40)	-	(mean)	36.9 (mean)	RS	IV	NFR
Delay [21]	2009	Retrospective cohort	880	30 (60)	850 (850)	-120	/	RS, OS, C	IV	NFR
Illouz [33]	2009	Prospective cohort	820	385 (770)	435	136 (mean)	45.6 (mean)	C, RS	IV	NFR
Veber [34]	2011	Retrospective cohort	31	31 (62)		(median)	38.16 (mean)	RS	IV	NFR
Wang [35]	2011	Retrospective cohort	48	48 (96)	-	18-72 (range only)	(mean)	RS	IV	NFR
Rubin [11]	2012	Prospective cohort	27	27 (54)	-	12 (mean)	35.9 (mean)	RS	III	NFR
Khouri [5]	2012	Prospective cohort	81	81 (162)	-	44 (mean)	Range only: 17-63	VR, RS	IV	NFR
Gentile [41]	2013	Retrospective cohort	100	35 (70)	65 (65)	12 (mean)	Range only: 19-60	VR, C, RS	III	NFR
Fiaschetti [37]	2013	Retrospective cohort	15	5 (9)	10 (13)	12 (mean)	46.27 (mean)	RS, VR	IV	NFR

Reference	Year	Study design	Total	Cosmetic Augmentation	Reconstruction	Follow up: months (mean/ median)	Age: years (mean/ median)	Reported outcomes	Level of Evidence (OCEBM))	Fundings
Auclair [38]	2013	Prospective cohort	197	197 (394)	-	12 (mean)	/	RS, VR	IV	NFR
Spear [36]	2014	Prospective cohort	10	10 (20)	-	12 (mean)	30 (mean)	VR, E, PS, RS, C	III	Funded by a grant from the ASERF[a]
Del Vecchio [39]	2014	Prospective cohort	30	27(54)	3 (3)	12 (mean)	/	VR	IV	NFR
Khouri [6]	2014	Prospective cohort	476	388 (776)	88	42 (mean)	37.6 (mean)	RS, VR, C	IV	NFR
Li [40]	2014	Prospective cohort	105	103 (206)	2	18 (mean)	31.3 (mean)	E, RS, C	IV	NFR
Chiu [10]	2014	Prospective cohort	282	282 (NS)	-	23.7/23 (mean)	33.1 (mean)	PS, RS, E, C	IV	NFR
Abboud[24]	2015	Prospective cohort	80	80 (160)	-	24 (mean)	42 (mean)	RS	IV	NFR
Uda[26]	2015	Prospective cohort	12	12 (12)	-	21 (mean)	43 (mean)	C, RS, VR	IV	NFR
Chiu[25]	2016	Retrospective cohort	27	27 (54)	-	27.1 (mean)	39.1 (mean)	C, VR, PS	IV	NFR

Abbreviations: ASERF, Aesthetic Society Education and Research Foundation; C, complications; E, esthetics; NFR, the authors received no financial support for the research, authorship, and publication of this article; OCEBM, Oxford Centre for Evidence-Based Medicine; OS, oncological safety; PS, patient satisfaction; RS, radiological safety; VR, volume retention.

Postoperative Management

Out of the 22 studies, 10 of them reported on postoperative management [4,5,21,24,30,31,33,39-41]. Two studies reported postoperative medicinal regiments [21,30], with both studies prescribing an unspecified kind of analgesic next to one study prescribing an unspecified kind of antibiotic and sleeping pill. Nine studies [4,5,24,30,31,33,39-41] reported using some postoperative protective or supportive types of breast garments, ranging in use from 1 day to 6 weeks. One study [41] reported using no garments, while one study [40] used local cold compresses only with suspected edema or inflammation. Two studies reported on the postoperative management of the donor site [21,24]. Of these studies, one used an abdominal support belt for 6 weeks next to endermology consultation when suspecting edema, and one study used compressive garments not further specified. Post-operative instructions were reported in three studies [21,39,40]. These instructions included harvest site massage instructions in one study and avoidance of breast compression for approximately 4 months, in the two additional studies [39,40].

Complications

In a total of 17 studies [4-6,10,21,24-26,29-31,36-38,40,41,43], an analysis showed an overall complication rate of 17.2% (95% CI 15.9-18.5) after a mean follow-up period of 34.5 months in 3409 patients after AFG for cosmetic purposes (Table 3). The following are complication rates over the total of patients from the studies that reported on that specific complication. Palpable indurations were seen in 33.3% (95% CI 20.4-46.3) of the cases [25,26,29]. Persistent pain was reported in one study [29] in 25.0% of the patients (95% CI 0.5-49.5). A hematoma was seen in 16.4% (95% CI 14.5-18.4) of the patients [4,30,33,37,38]. New nodules were reported in 11.0% (95% CI 8.6-13.4) of the cases, and cytological analyses of the aspirated or surgically removed material showed fat necrosis in all cases [5,6,36,40]. In 8.3% (95% CI 0.0-42.9) of the patients, abnormal breast fluid, lymphadenopathy, and pus discharge were seen [29]. Other complications were dysesthesia in 7.7% (95% CI 3.8 11.6) [4], fat necrosis in 6.6% (95% CI 5.5-7.7) [4,6,10,21,25,31,36,40], and calcifications in 4.5% (95% CI 2.8-6.6) [10,25,40] of the cases. Striae of the breast were seen in 4.3% (95% CI 3.0-5.6) [30,33] and cyst formation in 3.3% (95% CI 1.9-4.7) of the cases [4,24,31,38,40]. Infection of the breast was seen and treated with oral antibiotics, drainage, and/or ice packing in 0.9% (95% CI (0.5-1.2) [6,10,21,24-26,29,33,37,38] of the cases, and donor site infection was seen in 0.6% (95% CI 0.0-3.9) of the cases [5,24]. Donor site deformation was seen in 0.4% (95% CI 0.0-2.3) [24,38] of the cases;

pneumothorax, another rare complication, was seen in two patients [6,21,24,37](0.1% [95% CI 0.0-0.5]), and one [44] of those patients was treated with pleural drainage.

Table 3: Complications: Overview of complications and management

Study	Year	Pat. #	Complications	Management
Fulton [30]	2003	65	Hematoma: 0/65, Fat emboli: 0/65 Striae of the breast: 2/65	Daily application of Tretinoin
Zheng [31]	2008	66	Fat necrosis/ cyst formation: 11/66	Extirpation 2/11 (HP: fat necrosis)
Hyakusoku[29]	2008	12	Palpable indurations 12/12 Abnormal breast fluid 1/12 Persistent pain 3/12 Infection 1/12 Lymphadenopathy 1/12 Pus discharge 1/12	NR
Zocchi [4]	2008	181	Hematoma 143/181, Dysesthesia 14/181, Liponecrosis 2/181, Microcyst 3/181	
Delay [21]	2009	880	Infections: 6/880 Intraoperative pneumothorax: 1/880 Fat embolism 0/880, Fat necrosis 26/880	Drainage, topical treatment, antibiotics, ice Pleural drain placement
Illouz [33]	2009	820	Striae: 36/820, Hematoma: 88/820 Infection 5/820	Antibiotics
Khouri [5]	2012	81	Donor site infection (mycobacterial): 1/81 Masses or nodules: 0/81	Oral antibiotics and minor incision and drainage
Gentile [41]	2013	100	Hematoma (Coleman group): 1/33	
Khouri [6]	2014	476	Infection: 7/476 Pneumothorax: 1/476 Fat necrosis: 90/476 , Breast nodules: 71/476	Antibiotics only Chest tube for one day
Fiaschetti [37]	2013	15	Infections: 0/15, Bleeding: 0/15, Hematoma: 0/15, Pneumothorax: 0/15, Postoperative complications (NS): 0/15	
Spear [36]	2014	10	Fat necrosis: 1/10 Pain: 0/10, Palpable lumps: 0/10	Radiographic follow up
Auclair [38]	2013	197	Infections: 0/197, Hematomas: 0/197, Asymmetry of graft take: 0/197 Cystic masses: 2/197 Donor-site deformity (medial thigh): 1/197	Aspiration (showing fat necrosis) Additional fat grafting
Li [40]	2014	105	New oil cysts: 0/105, Fat necrosis: 0/105, Breast masses: 0/105, Benign-appearing calcifications: 2/105 Small nodules 3/105	Aspiration (revealing Fat necrosis)
Chiu [10]	2014	282	Fat necrosis: 3/282 (2 in group A, one in group B) Infection: 2/282 (1 in both groups) Induration and/ or calcification: 15/282 (10 in group A, 5 in group B)	

Study	Year	Pat. #	Complications	Management
Abboud[24]	2015	80	Cystic masses: 5/80 Infection: 2/80 Pneumothorax: 0/80 Fat Embolism: 0/80 Donor site complications NS: 0/80	Conservatively, with aspiration in one patient Oral Antibiotics
Uda[26]	2015	12	Palpable induration: 0/12 Infection: 0/12	
Chiu[25]	2016	27	Induration and/or calcification: 5/27 Fat Necrosis: 1/27 Recipient site infection: 1/27	NR

Radiological Follow-Up

Radiological images after AFG were studied in 19 articles (Table 4) [4-6,10,21,24,26,30-32,34-38,40,41,43,45]. One study was excluded because it reported the outcomes per image instead of the outcomes per patient [11]. The majority of the studies used standard pre- and postoperative mammograms to report on the radiological images (11 studies, n=1912) [4-6,21,24,30,32,34,35,37,38], with three studies (n=692) reporting Breast Imaging Reporting and Data System (BIRADS) outcomes only [26,33,36]. Some studies looked also at sonograms (4 studies, n=544) or MRIs (5 studies, n=305). Fat necrosis was reported in 14.0% (95% CI 11.4-16.6) of the cases on mammogram [4,6,37], 5.7% (95% CI 2.9-8.5) on sonogram [4,31,37], and 7.7% (95% CI 4.0-11.4) on MRI [5,37,40]. Cysts were seen on mammogram in 12.3% (95% CI 10.5-14.1) [4,21,24,32,34,38] of the cases, on sonogram in 5.1% (95% CI 2.0-8.2) [4,37] of the cases, and on MRI in 1.7% (95% CI 0.0-3.4) of the cases [26,37,40,41]. Micro- and macro-calcifications were seen on mammogram in 9.0% (95% CI 6.4-11.5) [4,5,24,32,34,35,37,38] and 7.0% (95% CI 3.8-10.2) [5,24,34,37,38], on sonogram in 4.8% (95% CI 2.8-6.7) [4,10] and 5.3% (2.7-7.9) [10], and on MRI in 1.9% (95% CI 0.1-7.3) [40] and 1.5% (0.3-4.5) [40,41], respectively, of the cases. Furthermore, the mammograms showed scar tissue in 12.9% (95% CI 1.1-24.7) [34] of the cases and benign calcification in 9.2% (95% CI 2.2-16.2) [30] of the cases.

Table 4: Radiological follow-up: Overview of physiological-/ mammographic-/ sonographic- and/ or MRI findings after AFG

Study	Year	Pat. # (breasts)	Timing of imaging	Follow up, months (mean/ median)	Physical Findings	Mammographic findings	Ultrasound findings	MRI findings	BI-RAD
Fulton [30]	2003	65	Postop annually	Up to 120 Mean 30.72	NR	Benign calcifications: 6/65, Suspicious lesions: 0/65	NR	NR	NR
Zheng [31]	2008	66	1 w, 1,3,6, 12 m postop	37 (mean)	Palpable mass 11/66	NR	Fat necrosis 11/66	NR	NR
Zocchi [4]	2008	181	preop + 1 y postop	12 (mean)	NR	Liponecrosis 2/181, Microcyst 3/181, Microcalcifications 7/181	Liponecrosis: 2/181, Microcyst: 3/181, Microcalcifications: 7/181	NR	NR
Carvajal [32]	2008	20	34.5 m postop (mean)	34.5 (mean)	NR	Microcalcifications: 9/20, Oil Cysts: 4/20, Lipid Cysts 4/20, Axillary lymph nodes 14/20, Axillary lymph node + Intramammary lymph node: 1/20, Heterogeneity of pectoral muscle density: 16/20	NR	NR	Mx: II: 17/20, III: 3/20, Of which 2/3 were reclassified as II 6 months later and 1 12 months later.
Delay [21]	2009	880	postop NS	Up to 120	NR	Oil Cysts: 132/880	NR	NR	NR
Illouz [33]	2009	670 (820)	6 m,1 y postop	136 (mean)	NR	BIRADS only	NR	NR	Mx: 0: 30/670, I: 315/670, II: 208/670, III: 117/670
Veber [34]	2011	31	16.2 m postop (mean, SD 13.5 m)	16.2 (median) SD 13.5	NR	Microcalcifications: 5/31, Macrocalcifications: 3/31, Cysts: 8/31, Scar tissue: 4/31	NR	NR	NR

Study	Year	Pat. # (breasts)	Timing of imaging	Follow up, months (mean/ median)	Physical Findings	Mammographic findings	Ultrasound findings	MRI findings	BI-RAD
Wang [35]	2011	48	18-72 m postop	18-72 (interval) Mean 22.9	NR	Microcalcifications in 8/48	NR	NR	NR
Rubin [11]	2012	27 (216 images)	12 m postop	12 (mean)	NR	Oil Cysts: 55/216, Scarring: 38/216, Calcifications benign/ fat necrosis: 37/216, Calcifications warranting biopsy: 10/216, Mass or distortion warranting biopsy: 6/216	NR	NR	Mx: I: 78/216, II: 114/216, III: 10/216, IV: 14/216
Khouri [5]	2012	81	Mx: 3, 6, 12 m postop (> 40 y of age only) MRI: 6, 12m	44 (mean)	NR	Micro-/ macrocalcifications: 12/81	NR	Fat necrosis: 12/81	
Gentile [41]	2013	100	Preop + 6, 12 m postop	12 (mean)	NR	NR	NR	Cystic formation + Macrocalcification: 1/33	NR
Fiaschetti [37]	2013	15 (24)	Preop + 3,6, 12 m postop	12 (mean)		Microcalcifications: 5/24 b, Macrocalcifications: 3/24 b, Liponecrosis: 3/24 b	Oil Cysts: 11/24, Cytosteatonecrosis: 3/24	Oily cysts: 1/24, Cytosteatonecrosis: 4/24.	NR
Auclair [38]	2013	35	Preop + 12 m postop	12 (mean)	NR	New calcifications: 0/35, cysts: 0/35, masses: 0/35	NR	NR	NR
Khouri [6]	2014	476	12 m postop	42	15% (71) of patients. All confirmed as benign	Fat necrosis 90/NS (only >40 yrs women)	NR	NR	NR

Study	Year	Pat. # (breasts)	Timing of imaging	Follow up, months (mean/ median)	Physical Findings	Mammographic findings	Ultrasound findings	MRI findings	BI-RAD
Spear [36]	2014	10	Preop + 12 m postop	12 (mean)	NR	BIRADS only	NR	BIRADS only	Mx: 0: 2/10, I: 3/10, II: 4/10, IV: 1/10 MRI: 0: 5/10, II: 5/10
Li [40]	2014	105	18 m postop	18 (mean)	Small nodules: 3/105	NR	NR	- New oil cysts: 0/105 - Fat necrosis: 0/105 - Breast masses: 0/105 - Benign-appearing calcifications: 2/105	NR
Chiu [10]	2014	282	3,6,12 m postop	23.7 (mean)	NR	NR	Micro-/ macrocalcification: 15/282	NR	NR
Abboud[24]	2015	80	6 m postop + annually	24 (mean)	NR	New Calcifications 0/80, Cysts: 0/80, Masses: 0/80	NR	NR	NR
Uda[26]	2015	12	Mx annually MRI > 6m postop	Mx 37 (mean) MRI 14 (mean)	NR	BIRADS only	NR	- Small cystic (non-palpable) regions: 2/12	I: 11/12 III: 1/12 (benign calcification on biopsy)

Abbreviations: BIRADS, Breast Imaging Reporting and Data System; MRI, magnetic resonance imaging; Mx, mammogram; m, month; NR, not reported; NS, not specified; postop, postoperative; preop, preoperative; pts, patients; r, range; SD, standard deviation; Sx, sonogram; w, week; y, year.

Fat Grafting Technique

Twenty-one articles described, to some extent, the methods of preparing and grafting the adipose tissue (Table 5) [4-6,10,11,21,24-26,30-41]. The anesthetic method was reported in 11 studies, with 8 studies using general anesthesia [4,11,21,24,30,37,40,41], 2 studies adding local anesthesia [10,25], and 1 study using just a local form of anesthesia [35]. The abdomen was the primary donor site in most studies. Fat from the gluteal area or the arms was used in cases of insufficient supply or an odd fat distribution. For harvesting, most authors described performing manual aspiration, usually with a 2- to 4-mm cannula attached to a 1- to 60-cc syringe. The majority of the studies applied centrifugation on 3000 rpm for periods ranging from 4 to 5 minutes. Four studies [4-6,26] combined AFG with the pre- and postoperative use of the BRAVA system, and one study [39] used only the device 3 weeks preoperatively. Five studies reported the use of supplements, which were composed of PRP [30,41] or SVF [10,25,45]. For the injections, most studies described using a multiplane, retrograde (on withdrawal) injection technique. The primary site of injection was the subcutaneous space with additional injections most often performed into the sub-pectoral and retroglandular spaces. The number of sessions for delivering AFG to achieve a satisfactory result was reported in 14 studies [4-6,24,26,30,31,33,34,36-40] and varied from one to three sessions (i.e., 1 session for 1190 patients, 2 sessions for 127 patients, and 3 sessions for 32 patients). No significant associations were found between the volume of the initial fat graft and the number of sessions or between the form of augmentation and the number of sessions.

Table 5: Fat grafting technique: Overview of the form of anaesthesia, donor site, infiltration solution, harvesting-, preparation-, and injection technique used. Addition of supplementation, rigotomies or overcorrection, the number of sessions and the injected volume are subsequently given.

Reference	# pat.	Anesth.	Donor Site	Infiltration	Harvesting	Preparation	Supplementation	Inject. technique	Rig.	Over Corr. (%)	No. sessions	Volume injected/ breast (cc)/ session (mean)
Fulton [30]	65	Gen	Th, Fl	TS	Manual	LRS washing + water/ oil removal	PRP (2x C/ 5600, 2400 rpm 400-500 cc blood)	Blunt triple-port cannula, multiplane (subpect., pect., retroglan.), retrograde manner	NR	NR	1	200-300
Zheng [31]	66	NR	Abd, Tr, Th	NR	vMA	NaCl washing + C/ 600 rpm for 2 min	NR	3 mm blunt cannulas, multiplane (subcutan., inframammary, subgl.) manner	NR	NR	17 (3), 21 (2), 28 (1)	60-120 subcut. (101), 60-90 subgl. (73)
Carvajal [32]	20	NR	Abd, back, Th, arms	NR	NR	NR	NR	Retrogland./ pect. injection	NR	NR	NR	150-300 (235)
Zocchi [4]	181	Gen	Tr, GM	NaCl/ Epi	Manual	Washing + vibration (30 sec)	BRAVA (30 days preop/ 12 h/day)	2 mm flex and stiff, curved cannulas, biplane (retrogland. subcut.) retrograde manner	NR	NR	1	160-745 (375)
Delay [21]	880	Gen	Abd, Th, KN, Tr	NR	Manual	C/ 3200 rpm for 3 min.	NR	Multiplane (deep- superficial) retrograde manner	NR	NR	NR	NR
Illouz [33]	820	NR	Abd. Fl, Th, KN	NaCl/ Epi	Manual	Decanting: 10-15 min.	NR	2,5 mm cannula, multiplane (subcut. and intraglan.) retrograde manner	NR	NR	1-5	25-180 (145)
Veber [34]	31	NR	NR	NR	CT	Coleman technique	NR	Multiplane (subcut., retropect.) manner	NR	NR	2 (3), 5 (2), 24 (1)	(200.8)
Rubin [11]	27	Gen	NR	NaCl/ Epi	MA	NR	SVF	18 G needle, multiplane (subcut., pect.), machine driven manner	NR	NR	NR	(267.2)
Wang [35]	48	Loc	NR	NR	Manual	NR	NR	2 mm cannulas, singleplane (retromammary) retrograde manner	NR	NR	NR	50-170
Khouri [5]	81	NR	NR	NR	vMA	C/ 15 g for 3 min.	BRAVA: preop, 10 h/day for 4 weeks, postop 24-72 h + nightly	2,4 mm blunt cannulas, multiplane (subdermal, subcut., subgland., pect., subpect.), machine driven, retrograde manner	NR	NR	1	(282)
Gentile [41]	100	Gen	Abd	NR	Manual	C/ 3000 rpm for 3 min	PRP vs AFG	1-2 mm cannulas, multiplane, retrograde manner	NR	NR	NR	(120.0)

Reference	# pat.	Anesth.	Donor Site	Infiltration	Harvesting	Preparation	Supplementation	Inject. technique	Rig.	Over Corr. (%)	No. sessions	Volume injected/ breast (cc)/ session (mean)
Khouri [6]	476	NR	NR	NR	Manual	C/ 15 g for 2 min	BRAVA: preop, 10 h/day for 2-4 wks, postop 3-4 wks	2.4 mm cannula, multidirectional manner until interstitial pressure of 9mmHg	Yes	NR	8 (3), 73 (2), 395 (1)	(329.3)
Fiaschetti [37]	15	Gen	NR	KS	NR	C/ 3000 rpm for 4 min	NR	Coleman microcannula, multiplane retrograde manner	NR	NR	2	(187)
Spear [36]	10	NR	Abd, Th	NR	MA	C/ 3000 rpm for 3 min	NR	Blunt cannula, multiplane (subcut., subglan., intramusc.) injection	NR	NR	1	90-324 (243)
Del Vecchio [39]	30	NR	Th	NaCl/ Epi	MA	C/ 30-40 g, for 3 min	BRAVA: 3 weeks preoperative (NS)	14 G Coleman needle, single plane (subcut.) manner	NR	NR	1	(606.7)
Auclair [38]	197	NR	NR	NR	MA	C/ 3000 rpm for 2 min	NR	1.5-1.6 mm cannula, multi plane (subcut., retrogland, around implant) retrograde manner	NR	NR	5 (2), 192 (1)	NR
Chiu [10]	282	Gen + Loc (NS)	NR	LRS/ Lido/ Epi	MA	C/ 800 g for 4 min	SVF	Multiplane (subcut., intramusc., retromusc., and premusc.) manner	NR	NR	NR	(247.5)
Li [40]	105	Gen	Abd, Fl, Tr, Th, KN, arm	NaCl/ Lido/ Epi	MA	NaCl washing 3-4x + cotton pad decanting	NR	14 G blunt cannula, multiplane (retro-/ intraglan., subcut.) manner	NR	NR	5 (3), 20 (2), 80 (1)	(205)
Abboud[24]	80	Gen	Fl, Th, Abd	KS	MA	C/ 3000 rpm and 0.7 atm, time NS	NR	Customised v-shaped multihole 3 mm cannula, multiplane (superficial, deep subcut., parenchymal, pericapsular, muscular and submusc.) manner	NR	Yes	80 (1)	300-600 (420)
Uda[26]	12	NR	1st session: Th, Abd/ GM, Wst	NR	NR	C/ 1200 g for 3 min	BRAVA: 10 hrs/day for 4 wks preop + 10 hrs/day for 2 weeks starting on postop day 1	Multiplane (subcut., subglan., intramusc.,submusc.) using microdroplet technique	NR	NR	9 (1), 3 (2)	100-350 (211)
Chiu[25]	27	Gen + Loc (NS)	Th, Hips, Fl, Abd, Cl	LRS/ Lido/ Epi	MA	C/ 800 g for 4 min	SVF	14 G single-hole cannula, multiplane (subcut., subglan., supramusc., intramusc.) manner.	NR	NR	NR	(247)

Abbreviations: abd, abdomen; atm, atmosphere; c, cold; C, centrifugation; Cl, calves; CS, Colemans solution; CT, Coleman technique; DS, deep sedation; epi., epinephrine; Fl, flanks; g, G-force; G, gauge; Gen, general anesthesia; gland., glandular; GM, gluteus region; h, hour; intraglan., intraglandular; intramusc., intramuscular; KN, knees; KS, Kleins solution; Lb, left breast; LD, latissimus dorsi; lido, lidocaine; LLS, Luer lock syringe; Loc, local anesthesia; LR, lumbar region;

LRS, lactate ringer solution; MA, machine assisted; NaBic, sodium bicarbonate; NAC, nipple areola complex; NaCl, sodium chloride; NR, not reported; NS, not specified; pect., pectoral; PG, puregraft; postop, postoperative; preop, preoperative; Rb, right breast; retrogland., retroglandular; retromusc., retromuscular; retrospect., retrospctoral; rpm, range per minute; Sed, analgesic sedation; subcut., subcutaneous; subgl., subglandular; subpect, sub-pectoral; supramusc., supramuscular; SVF, stromal vascular fraction; Th, thighs; Tr, trochanteric region; TS, tumescent solution; vMA, vacuum machine assited; Wst, waist.

Volume Retention

In addition to patient satisfaction, objective measurements of the volumetric result, by way of a reliable volumetric analysis, are imperative to demonstrate the efficacy of AFG. Recently, more sophisticated ways of measuring have been used, such as specified 3D measuring systems or MRI analyses. Eight studies with a total of 523 patients complied with the inclusion criteria of sufficient sample size and follow-up period and were included in the final analysis (Table 6) [5,6,25,26,30,36,38,39]. Four studies [5,6,26,36] used MRIs, and three studies used advanced 3D measuring systems; one study combined an MRI and 3D measurements [36,38,39], and one study used volumetric measurement [30]. Additionally, one study measured volume retention through the difference in breast thickness, by way of a sonogram, at the 3 o'clock and 9 o'clock peri-areolar points between implant removal and 1 year after additional AFG [25]. Five of the eight studies (n=419) [5,6,36,38,39] described a mean total preoperative volume of 225.26 mL. The mean total injected volume per breast was 339 cc in seven studies (n=458) [5,6,25,26,36,38,39] and the mean volume gain per breast as described in six studies (n=431) [5,6,26,36,38,39] was 216.2 cc over a minimal period of 1 postoperative year. The retention of injected fat over a total of eight studies was 62.4% (range, 44.7-82.6%), with an average follow-up period of 16.6 months (range, 12-120). When correcting for important technical variables like preparation and the use of supplementation, there was 60.9% volume retention in the seven studies [5,6,25,26,36,38,39] (n=458) that used centrifugation (range, 15-1200 g or 3000 rpm for 2-4 min) as form of preparation. Furthermore, a 67.9% retention rate was found in the four studies [5,6,26,39] (n=401) that used the BRAVA system pre- and postoperatively. Due to the heterogeneity among the studies regarding the description of preparation, supplementation, and injection technique, no association could be found concerning volume retention. However, regarding the harvesting technique, most studies reported using either a manual [6,30] (2 studies, n=541) or machine-assisted [5,25,36,38,39] (5 studies, n=355) form of aspiration with volume retentions of 79.0% and 61.0%, respectively (P=<.0001).

Table 6: Volume Retention: Overview of the auxiliary method, the method of measuring volume retention, the indications and number of sessions as well as the mean total injected volume, the volume gain and the percentage of gain relative to the injected volume.

Study	Year	Auxiliary method	Meth. of measuring	Indication	Pat. #	Mean preoperative breast volume (ml)	Sessions	Mean total injection volume/breast (ml)	Mean volume gain/ breast (ml)	Volume gain relative to graft volume (%)	Follow-up (months)
Fulton [30]	2003	PRP	VM	Cosmetic	65	NR	1	200-300	NR	73 (r 20-140)	-120
Khouri [5]	2012	BRAVA	MRI	Cosmetic	71	371	1	282	233 (r 60-619)	82.6	12
Auclair [38]	2013	Implants	3D	Cosmetic	20	164.4	NR	368	204.9	56.1	12
Spear [36]	2014	NR	MRI/ 3D	Cosmetic	10	75.3 (r 48.6–125.5)	1	236 (r 90-324)	105.5 (r 63.8-186.8)	44.7	12
Del Vecchio [39]	2014	BRAVA	3D	Cosmetic	24	196.6	1	610.0	305.8	50.1	12
Khouri [6]	2014	BRAVA	MRI	Cosmetic	294	319	8 (3), 73 (2), 395 (1)	367	293	79.8	42
Uda[26]	2015	BRAVA	MRI	Cosmetic (symmetrisation unaffected breast after reconstruction)	12	NR	3 (2), 9 (1)	263 (r 150-560)	155.17	59.0	14
Chiu[25]	2016	SVF	Sonogram	Cosmetic (AFG for implant removal)	27	NR	27 (1)	247	NR	54.0 [a]	12

Abbreviations: 3D, three-dimensional; BRAVA, breast enhancement and shaping system; CT, computer tomography; I-Fc, implant-to-fat conversion; MRI, magnetic resonance imaging; NR, not reported; PRP, platelet-rich plasma; r, range; SVF stromal vascular fraction; VM, volumetric.

[a]: This percentage was a calculation of the difference in breast thickness (measured in millimetres at 9 o'clock and 3 o'clock direct peri-areolar) measured by sonogram directly after implant removal compared to the thickness measured at the last follow-up examination.

Patient/Surgeon Satisfaction

A total of six studies [4,10,25,31,36,40] reported on patient and/ or surgeon satisfaction on a 3- to 5-point Likert scale which were manually converted to a 3-point-Likert scale using the conversion model described in Appendix A (available as Supplementary Material at www.aestheticsurgeryjournal.com). Three (n=529) and four studies (n=463) reported patient and surgeon satisfaction after AFG, respectively, over a mean follow-up period of 1 year. Patient satisfaction was achieved in 92%, and 89% of the surgical teams reported a good result on post-operative photograms or clinical assessments (Table 7).

Table 7: Patient/ Surgeon satisfaction

Study	Year	No. of patients	Follow up: months	Patients satisfaction	Surgeons Satisfaction Measurement:	
Zheng [31]	2008	66	37 (mean)	Satisfied 80.3%, Neutral 0%, Dissatisfied 19.7%	PPoPc/ three IS	Good 78.8%, Neutral 0%, Poor 21.2%
Zocchi [4]	2008	181	12 (mean)	Satisfied 91.7%, Neutral 5.5%, Dissatisfied 2.8%	NS	Good 80.7%, Neutral 13.8%, Poor 5.5%
Spear [36]	2014	10	12 (mean)	NR	PPoPc/ 14 (BO) (NS)	Good 10%, Neutral 50%, Poor 40%
Li [40]	2014	105	18 (mean)	NR	Three IS (NS)	Good 83.8%, Neutral 0%, Poor 16.2%
Chiu [10]	2014	282	23.7/23 (mean)	Satisfied 85.5%, Neutral 0%, Dissatisfied 14.5%	PPoPc/ one IP	Good 85.8%, Neutral 0%, Poor 14.2%
Chiu[25]	2016	27	27.1 (mean)	Satisfied (r 67-100%): 27/27 Neutral (r 33-66%): 0/27 Dissatisfied (r 0-33%): 0/27	PPoPc/ one IP	Good (r 67-100%): 27/27 Neutral (r 33-66%): 0/27 Poor (r 0-33%): 0.27

Abbreviations: BO, blinded observers; IP, independent physician; IS, independent surgeon(s); NR, not reported; PPoCc, pre-/postoperative clinical comparison; PPoPc, pre-/postoperative photo comparison; PoAo, postoperative analysis only.

Risk of Bias Across Studies

A comprehensive overview of the risk of bias across the studies is given in Table 1.

Discussion

We aimed to give a comprehensive overview of the available evidence on the employed techniques and outcomes of AFG in cosmetic breast augmentation. As previously stated, the authors recently published a systematic review using the same methodology but reporting on AFG in addition to onco-plastic breast reconstruction [28]. The latter focuses specifically on the (oncological) safety and efficacy of AFG following various reconstructive techniques, such as myocutaneous flap- and prosthetic reconstruction as well as correction of contour deformities. In this systematic review, following approximately 3400 patients, during a mean follow-up period of 34.5 months, an overall total complication rate of 17.2% (95% CI 15.9-18.5) was found after breast augmentation with AFG. These results are similar to reports of studies with a follow-up period of up to 9 years after implant-based augmentation procedures [46]. However, safety is not only a matter of direct postoperative complications because long-term alterations in breast morphology can present a serious challenge in differentiating benign anomalies from malignancies on radiological examinations. Findings on mammograms, sonograms, and MRIs after AFG treatment appear similar to those findings after other forms of breast surgery [11,47,48]. Benign irregularities consisted of cysts, fat necrosis, calcifications, and scar tissue. Cysts are best differentiated from solid masses by way of a sonogram [49] and are described as oval hypoechoic findings, anechoic points, and anechoic areas with regular walls [43] An MRI is the best method to detect fat necrosis and differentiate it from oil cysts. It is decribed as heterogeneously hyperintense (appearing lighter in color than surrounding tissues) on T2 weighted images (water=bright/fatty content= dark) [50,51]. The presence of decreased signal intensity in the center of fat necrosis is key to differentiating it from a cancerous tumor, which, contrary to fat necrosis, can have a necrotic center [52,53]. Calcifications after AFG are easily seen on mammograms as white calcium deposits located in the wall of cysts or as coarse irregular spots, sometimes surrounding radiolucent areas of fat necrosis. These benign features are generally easily differentiated from malignant clusters of pleomorphic micro-calcifications on the basis of morphology, size, and distribution [54-56]. Furthermore, these findings seem to agree with recent large cohort

studies [4,11,21,33,34,47,48] that showed that most benign irregularities were easily distinguishable from malignancies as long as good communication exists between the surgeon and radiologist. Fortunately, radio diagnostic techniques and corresponding radiologist experience are continuously improving, enabling radiologists to interpret these findings more and more accurately. The efficacy of the technique is assessed by retention of the inserted volume and by the satisfaction with the results reported by the patient and surgeon. Eight articles reported an average volume retention of 62.4% (range, 44.7%-82.6%) after a mean follow-up period of 16.6 months. However, it should be noted that six of these articles used an auxiliary method for achieving higher volume rates, as well as retention, which can create a reporting bias since these results are not representative of the volume retention after the solitary use of AFG. Furthermore, the higher volume retention seen after manual aspiration should be interpreted with caution because substantial confounding variables exist. The reported satisfaction was considered high; on average, 92% of the patients and 89% of the surgeons were satisfied with the results. These satisfaction rates after a 1 year follow-up period and in a small cohort of patients seem to surpass those reported after implant-based augmentation procedures [57]. The following two recently published articles are especially worth mentioning in regard to the AFG technique: 1) systematic review by Strong et al. [2], which showed higher retention rates with centrifugation and slow injection of fat and 2) special topic article by Zielins et al. [58], which highlights the latest in vitro, as well as in vivo, findings regarding important steps in the AFG process. In summary, the authors described the perception of a three-zone survival system (ie, surviving, regenerating, and necrotic) when it comes to fat graft survival as previously described by Eto et al. [59]. The highest yield of AFG volume results from the survival of the regenerating zone, which brings forth ASCs with the potential for differentiation and replacement of "losses" in the necrotic zone, as well as the increased survival through the enhancement of revascularization. Furthermore, these ASCs, as well as the actual adipocytes, seem to thrive on the use of larger cannula sizes for harvesting, as well as injection (5-6 mm), than was previously reported by the ASPS Fat Grafting Task Force (3-4 mm) [60]. Another interesting aspect of AFG, as it was thought to make little difference in aesthetic outcomes [61,62], is the importance of the donor site location. Saint-Cyr et al. [63] recently reported better volume retention of trochanteric harvested fat, which they attributed to higher numbers of adipocytes and so called "colony forming units." With all of these different aspects, as well as external factors like supplementation (ie, PRP/ SVF)

and auxiliary methods (ie, BRAVA system) affecting the outcome, AFG remains a well-studied topic in which much information is yet to be discovered. However, despite all of these advancements, we should remain cautious because several experimental studies still show the potential danger of the interaction between adipose-derived stem cells and mammary epithelial cells, as well as the potential of CD34+ progenitors in white adipose tissue, to promote cancer stimulation/progression [45,64-67].

Limitations

This systematic review has several limitations. Reported evidence on the outcomes of AFG in cosmetic breast augmentation is still scarce. Only low-level studies (OCEBM III/IV) and mainly retrospective studies without a control group were found. The use of validated measurement tools to assess patient-reported outcomes is lacking, and data on oncological outcomes are absent. Heterogeneity between studies in reported outcomes and nomenclature regarding radiological findings and complications makes it difficult to draw conclusions. This was partly resolved by combining similar terms under one common nominator (eg, oil cysts and lipid cysts), but this may have introduced some bias. The mean volume retention in this review is the outcome of the reported percentages of the remaining volume after the follow-up period in the different studies. The heterogeneity between the studies in calculation of this volume retention can, however, cause a reporting bias. It should also be noted that several studies [6,21,33,37,39-41] report outcomes over a mixed cohort of patients without differentiating the outcomes based on indication. Finally, some articles report outcomes over a total cohort of both cosmetic and reconstructive patients. Both factors can independently cause a reporting bias. Therefore, since a systematic review can only be as strong as the articles it includes, certain caution is appropriate when interpreting these results. The aim of this systematic review was to complement the already broad knowledge base on the subject of AFG in cosmetic breast surgery. The authors believe this systematic review accomplishes that by the addition of three recently published studies [24-26], as well as the exclusion of case series/ reports and studies with insufficient follow-up periods (specifically for the AFG technique).

Conclusions

This review provides an updated overview of the important outcomes of AFG for cosmetic breast surgery. Although the evidence is still limited, AFG seems to be a promising method to achieve cosmetic breast augmentation with encouraging volume retention and satisfaction rates in a small number of studies. Complication rates and radiological findings are comparable to those after implant-based augmentation. However, good-quality RCTs are needed to compare augmentation techniques, grafting methods, and use of auxiliary methods to further assess safety and identify which factors affect the outcomes. Also, larger cohorts and longer follow-up periods are necessary to focus on cancer occurrence and detection to further substantiate the safety of this technique. Finally, more objective questionnaires, such as the BREAST-Q [68], are essential to evaluate patient satisfaction in breast surgery.

Supplementary Material

This article contains supplementary material located online at www.aestheticsurgeryjournal.com.

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Chapter 4

The use of Autologous Fat Grafting for the treatment of scar tissue and Scar-Related Conditions: A systematic review

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Abstract

Background: Scar tissue can cause cosmetic impairments, functional limitations, pain, and itch. It may also cause emotional, social, and behavioral problems, especially when it is located in exposed areas. To date, no gold standard exists for the treatment of scar tissue. Autologous fat grafting has been introduced as a promising treatment option for scar tissue-related symptoms. However, the scientific evidence for its effectiveness remains unclear. This systematic review aims to evaluate the available evidence regarding the effectiveness of autologous fat grafting for the treatment of scar tissue and scar-related conditions.

Methods: A systematic literature review was performed using MEDLINE, Cochrane Library, EMBASE, and Web of Science. No language restrictions were imposed.

Results: Twenty-six clinical articles were included, reporting on 905 patients in total. Meta-analysis was not performed because of the heterogeneous methodology demonstrated among the articles. Main outcome measures were scar appearance and skin characteristics, restoration of volume and/or (three-dimensional) contour, itch, and pain. All publications report a beneficial effect of autologous fat grafting on scar tissue. There is statistical significant improvement of the scar appearance, skin characteristics, and pain. Itch and restoration of volume and three-dimensional contour also improved.

Conclusions: Autologous fat grafting is used to improve a variety of symptoms related to scar tissue. This systematic review suggests that autologous fat grafting provides beneficial effects with limited side effects. However, the level of evidence and methodological quality are quite low. Future randomized controlled trials with a methodologically strong design are necessary to confirm the effects of autologous fat grafting on scar tissue and scar-related conditions.

Introduction

Every injury to the dermis heals to form a scar. Dermal scars vary considerably with regard to appearance, form, stiffness, and contour, depending on the injury and the characteristics of the wound healing process [1]. Although scar tissue is necessary for the final stage of wound healing, it can have several adverse consequences. Scars can be cosmetically disfiguring, and severe scarring can cause emotional, social, and behavioral problems. For instance, Levine et al. [2] concluded that patients with facial trauma report higher levels of depression and anxiety, and they feel dissatisfied with their body image. Scars can also induce chronic pain, which may develop after acute pain, a symptom of normal wound healing. Another symptom is itch, producing severe discomfort and causing patients to scratch, which, in turn, threatens the healing process [3–5]. Furthermore, hypertrophic or keloid scars, contractures, and adhesion formation can cause functional limitations [6]. A survey by Young and Hutchison [7] showed that many patients are unhappy with a scar that results from surgery, and more than 90 percent would appreciate any improvement of this scarring. For the treatment of scars, clinicians and researchers have described a variety of protocols. Yet, limited data about the effectiveness are derived from well-designed, prospective, randomized controlled clinical trials. To date, no gold standard exists for the treatment of scar tissue. Treatments are mostly based on individual experience of clinicians, with varying degrees of success [8,9]. A relatively new option for the treatment of scar tissue is the use of autologous fat grafting, first described by Neuber [10] in 1893 and later refined by Coleman [11]. Autologous fat grafting has a volume increasing effect and is thought to stimulate the neosynthesis of collagen fibers, which therefore increases the dermal thickness, resulting in an improvement of skin quality [12–15]. It has also shown improvement of different types of pain. The hypothesis is that mesenchymal cells of the graft give prolonged analgesia by changes in the microenvironment and secretion of substances [16–18]. The use of autologous fat grafting is used increasingly in common practice, but to date, there is a lack of scientific evidence regarding the effects on scar tissue [13]. Our goal is to systematically review the available literature that describes the effectiveness of autologous fat grafting in the treatment of scar tissue-related symptoms, including the appearance of the scar, skin characteristics, restoration of volume and/or (threedimensional) contour, pain, and itch. In the present review, the term autologous fat grafting is used for all procedures that transplant

autologous fat to different parts of the body, such as lipofilling or fat transplantation.

Materials and methods

Literature Search

This systematic review was performed in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines [19] and in narrow collaboration with a medical librarian of the VU Medical Centre. The literature review was conducted using MEDLINE, Cochrane Library, EMBASE, and Web of Science databases. The keywords used for the search are shown in Table 1. All original articles were categorized manually according to the medical indication. From this databank, original articles that describe autologous fat grafting for the treatment of scar tissue and scar-related conditions, with or without the use of supplements, were found eligible for inclusion. The references of retrieved articles were screened to identify other potentially relevant articles.

Table 1. Keywords used for the search

Fat	- Grafting (graft*)
Lipo	- Transplantation (transplant*)
Adipocyte	- Transferring (transfer*)
Lipocyte	- Filling (fill*)
	- Harvesting (harvest*)
	- Augmenting (augment*)
	- Plasty (plast*)
	- Injection (inject*)
	- Infiltration (infiltrat*)
	- Sculpting (sculpt*)
	- Modelling/ modification (mod*)

Selection of Studies

Citations were title and abstract reviewed for eligibility by two independent reviewers (V.L.N. and J.-W.G.), and in case of disagreement, they were reviewed by a third researcher (M.G.M.). The full text was retrieved for evaluation of final inclusion (Fig. 1).

Inclusion Criteria

Randomized controlled trials, prospective and retrospective cohort studies, and case-control studies published between January of 2004 and August of 2014 were included. The articles needed to describe the applications of autologous fat grafting in relation to the treatment of scar tissue-related symptoms, whether or not in combination with a supplement or laser therapy. Other criteria were a clear description of the indications, goals, and clinical relevant outcomes. The design of the studies was based on the definition described by Dekkers et al.[20] The level of evidence of the studies was scored independently by two authors (V.L.N. and J.-W.G.) according the Oxford Centre for Evidence-Based Medicine 2011 levels of evidence.[21]

Data Extraction

The two reviewers independently extracted data from each article. When numbers of a specific endpoint were not provided in the article, an attempt was made to contact the authors for more information or to clarify the results.

Exclusion Criteria

Exclusion criteria were fewer than five patients, a follow-up period of less than 6 months, animal studies, and in vitro studies. There were no language restrictions. For the translation of the non-English articles, Google Translate was used.

Statistical Analysis

The extracted data are summarized in Tables 2 through 6. Meta-analysis was not performed because of the heterogeneous methodology demonstrated among the articles.

Results

In total, 17,956 articles were screened based on title (Fig. 1); 26 studies of 905 patients were included. Autologous fat grafting is used as treatment for a variety of symptoms related to scar tissue, including the appearance of the scar and

skin characteristics (Table 2) [22–27], restoration of volume and/or (three-dimensional) contour (Table 3) [25,28–39] pain (Table 4) [26,27,40–44] and itch (Table 5) [26,27,43]. Autologous fat grafting is also used in combination with other treatments (Table 6) [39,45–47]. The levels of evidence were II in five studies [33,36,39,41,45], III in seven studies [23–26,38,42,44] and IV in 14 studies [22,23,28–30,32,34,35,37,40,43,46–48]. Four randomized controlled trials [33,36,39,45], 16 prospective cohort studies [22,25–27,29,30,32,34,35,37,38,41–44,46] and six retrospective cohort studies [23,33,36,39,45,47] were included. Seven studies showed a statistically significant result [25–27,33,41,42,44] and 19 studies did not report any statistical analyses [22–24,28–32,34–40,43,45–47].

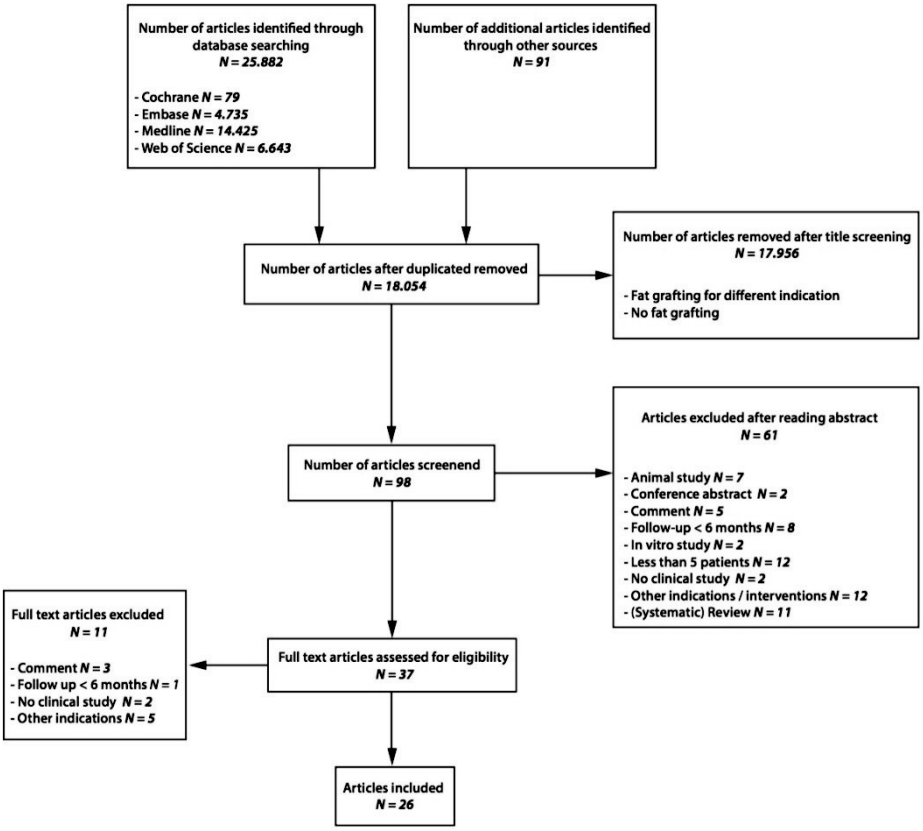

Figure 1: Diagram of article selection

Supplements

Several studies described the use of a supplement added to the fat graft. The following supplements were used in the articles included in this review.

- Platelet-rich plasma: Platelet-rich plasma has a platelet concentration above baseline, specifically 1,000,000 platelets per microliter. The α-granules of platelets release growth factors, which stimulate cell proliferation and cell differentiation for tissue regeneration [49,50].
- Adipose-derived stem cells/stromal vascular cell fraction: Aspirated fat can be used as the cell source of stromal vascular cell fraction, which contains adipose-derived stem cells. The survival rate of transplanted fat is better when transplanted with adipose derived stem cells [51].
- Basic fibroblast growth factor: Basic fibroblast growth factor is a potent mitogenic factor for adipocytes; it induces growth of new fat cells, whereas mature fat cells remain viable [52,53].
- Insulin: Multiple injections of insulin are associated with local lipohypertrophy and an increase of the adipocyte area percentage [54,55].

Laser Treatment

Two articles described the use of autologous fat grafting in combination with laser therapy. Cervelli et al. [45] used a fractional nonablative laser, and Nita et al. [46] used a fractional carbon dioxide laser. The combination of autologous fat grafting with laser treatment seems to have a synergistic effect on the treatment of scar tissue [56].

Procedure

Seventeen of the included articles used autologous fat grafting according to the Coleman technique in 663 patients [23–27,29,30,32,35,37,40–42,44–47]. Although more procedures maintain the histologic structure, Pu et al. [57] described that the Coleman technique creates a higher percentage of viable adipocytes and sustains a more optimal level of cellular function within the harvested fat grafts. Gentile et al. [25] described the use of stromal vascular cell fraction–enhanced autologous fat grafting according to the Coleman technique. The authors reported approximately 250,000 [(±) 34,782] nucleated cells per milliliter of fat tissue by manual extraction [25,58]. Sterodimas et al. [36] used adipose-derived stem cells and extensively described the process of isolation by enzymatic digestion, filtration, and centrifugation of the stromal vascular cell fraction.

Scar Appearance and Skin Characteristics

The effect of autologous fat grafting on the appearance of scar and skin characteristics was described in six studies that included 190 patients (Table 2) [22–27]. After a 12-month follow-up, there were significant improvements of dermal elasticity in a group of 14 patients [27], scar stiffness and thickness in 38 patients [26,27], skin hardness, scar color, mobility, vascularization, pigmentation, pliability, relief, and overall result evaluated by patient and observer in 20 patients [26]. A total of 156 patients reported a general satisfaction with the outcome of the procedure [22–25], which was confirmed by the surgeon [23] or an independent observer [22,24].

Table 2: Studies that report on outcomes using improvement of scar appearance and skin characteristics

Author	Design	N	FU	Indication	Therapy/ comparison	Methods	Results	OCEBM
Brongo et al., 2012 [22]	PCS	18	12	Severe burn outcomes	AFG	Patient satisfaction (0 to 10) Pre- and postoperative digital photographs assessed by surgeons and independent medical observer	General satisfaction score of 7.5 Better texture, softness, thickness, color and elasticity and reduction of retraction were observed	IV
Bruno et al., 2013 [24]	PCS	93	6	Burn scars	AFG[c]	Appearance, symptoms, awareness, satisfaction in appearance and satisfaction about the symptoms (patients questionnaire: 28-112)	Subjective appearance increase from 31 (range, 26-38) to 95 (range, 81-102)	III
						Vascularization, pigmentation, pliability, thickness and relief on the skin surface (Objective evaluation: 5-50)	Vancouver scale decrease from 41 to 15	
Gentile et al., 2014 [25]	PCS	30	12	Burns sequelae	SVF-enhanced AFG[c] (10) vs AFG[c] + PRP (10) vs AFG[c] (10)	Patients self-evaluation	All the patients were satisfied with the resulting texture and softness	III
Klinger et al., 2013 [26]	PCS	20	12	Painful and retractile scars	AFG[c] vs saline solutions (scar divided into two parts)	Scar color, hardness, thickness, impaired movements and overall evaluation by patient (POSAS) Scar vascularization, pigmentation, pliability, thickness, relief and overall evaluation by observer (POSAS)	Reduction of parameters[a] compared with baseline	III
						Durometer measurements (skin hardness)	Reduction of skin hardness[a] (mean value preoperative 33.75 (control) vs 40.91 (case area) and postoperative 30.72 (control) and 31.6 (case area) compared with control group	

Author	Design	N	FU	Indication	Therapy/ comparison	Methods	Results	OCEBM
Sardesai et al., 2007 [27]	PCS	14	12	Facial scars	AFG[c]	Scar color, scar stiffness, scar thickness and scar irregularity (POSAS: Patient Scale)	Improvements of scar stiffness and scar thickness[a] Improvement of other parameters (not significant)	III
						Quantitative assessment with the Cutometer SEM575 skin elasticity meter and the Derma-Spectometer	Improvement in dermal elasticity[a] No significant differences in vascularity or pigmentation	
Viard et al., 2011 [23]	RCS	15	66	Facial burns sequel	AFG[c]	Patient and surgeon satisfaction (3-point scale) of mimic features, skin texture and thickness	Good result in 13 patients (86%) and acceptable result in 2 patients (14%)	IV

Abbreviations: Significant result; $p < 0.05$ (a) , AFG according to Coleman's technique (c), Autologous Fat Grafting (AFG), mean Follow Up in months (FU), Number of patients (N), Not Reported (N.R.), Oxford Centre for Evidence-Based Medicine 2011 (OCEBM), Platelet-rich Plasma (PRP), Patient and Observer Scar Assessment Scale (POSAS), Prospective Cohort Study (PCS), Randomized Controlled Trial (RCT), Retrospective Cohort Study (RCS), Stromal Vascular cell Fraction (SVF).

Restoration of Volume and (Three-Dimensional) Contour

In 13 articles, authors described autologous fat grafting for improvement of volume and/or (three-dimensional) contour in relation to scarring for 357 patients (Table 3) [25,28–39]. Volume retention was assessed from preoperative and postoperative photos. There was a volume retention of 31 percent in 10 patients treated with autologous fat grafting [38]; 39 percent in 10 patients treated with autologous fat grafting according to the Coleman technique; 63 percent in 10 patients treated with stromal vascular cell fraction-enhanced autologous fat grafting according to the Coleman technique [25]; 70 percent with autologous fat grafting + 40 percent or 50 percent platelet-rich plasma (29 patients) [38,39]; and 90 percent if treated with autologous fat grafting + 40 percent platelet-rich plasma + insulin (10 patients) [39]. Follow-up of these studies ranged from 12 to 18 months. Volume retention was evaluated with three-dimensional breast imaging by a computed tomography scan in two studies that included 25 patients. Reported resorption rate ranged from 44 percent to 47.5 percent after a follow-up of 9 months to 3 years. Absorption rate estimated by patients was 53 percent [30,37]. A total of 188 patients were satisfied with the results, and only four patients reported moderate results [25,28–31,34,36] There were good results in 130 patients assessed by the surgeons [29,31,32,35], as well as in 19 patients evaluated by an independent assessment [28]. A moderate result was reported in 23 patients [31,34,35].

Table 3: Studies that report on outcomes using volume and/or (three-dimensional) contour restoring

Author	Design	N	FU	Indication	Therapy/ comparison	Methods	Results	OCEBM
Amar et al., 2007 [37]	PCS	15	9	Seguelae after conservative surgery for breast cancer	AFG[c]	Estimated percentage of resorption by patients	Estimated rate of resorption: 52.6%	IV
						3D breast imaging by a CT scan	Rate of fat resorption (CT-scan): 47.5%	
Arcuri et al., 2013 [28]	RCS	19	12	Posttraumatic maxillofacial deformities	AFG	Virtual Ideal Face (VIF) compared with postoperative photograph: patients evaluation	All patients were satisfied with the postoperative facial morphology	IV
						Evaluation by six blinded maxillofacial surgeons (5 points scale)	Similar facial morphology in 16 patients (84%): Scores of ≥ 3 (similar, very similar or identical)	
Baptista et al., 2013 [29]	PCS	20	18	Sequelae of rhinoplasty (dorsum irregularities, inverted V deformations, visible lateral osteotomies and saddle nose deformity)	AFG[c] (10) or AFG (10)	Patient satisfaction en clinical evaluation (3-point scale based on pre- and postoperative photos)	Patients: Satisfied or very satisfied in 18 cases Clinical evaluation: 14 very satisfactory cases and 3 satisfactory cases	IV
Beck et al., 2011 [30]	PCS	10	36	Sequelae of conservative surgery for breast cancer	AFG[c]	Patient satisfaction (score 0-10)	Mean patient satisfaction score: 6 (0-10) Estimated rate of resorption: 53%	IV
						3D breast imaging by a CT-scan	Rate of fat resorption (CT-scan): 44%	
Cervelli et al., 2009 [38]	PCS	35	18	Multiple facial applications	PRP + AFG (25) vs AFG (10)	Objective evaluation based on pre- and postoperative photos	70% maintenance of contour restoring and 3D volume in group treated 50% PRP + AFG, 31% in group treated with AFG	III

Author	Design	N	FU	Indication	Therapy/ comparison	Methods	Results	OCEBM
Cervelli et al., 2012 [39]	RCT	39	12	Soft tissue defects and scars	AFG + PRP (29) vs AFG + PRP + insulin (10) vs control group (number and treatment not reported)	Objective evaluation based on pre- and postoperative photos	35% volume maintenance in control group 70% volume maintenance in AFG + 40% PRP 90% volume maintenance in AFG + 40% PRP + insulin	II
Delay et al., 2007 [31]	RCS	42	20.6	Sequelae after conservative surgery for breast cancer	AFG	Patient satisfaction (4-point scale)	38 patients were satisfied or very satisfied	IV
						Clinical evaluation + 3D imaging assessed by 2 surgeons (4-point scale)	Good or excellent result in 38 patients	
Gentile et al., 2014 [25]	PCS	30	12	Burns sequelae	SVF-enhanced AFG^{c} (10) vs AFG^{c} + PRP (10) vs AFG^{c} (10)	Patients perception of contour restoring	All the patients were satisfied with the resulting contour	III
						Team evaluation based on pre- and postoperative photographs (6-point scale)	Contour restoring and 3D volume of 69% in patients treated with AFG^{c} + 50% PRP, 63% with SVF-enhanced AFG^{c,} and 39% with AFG^{c} alone	
						MRI and ultrasound	Lower fat resorption in reconstruction with SVF-enhanced AFG^{c} and AFG^{c} + PRP	
Guisantes et al., 2012 [32]	PCS	8	18	Retractile and dystrophic scars	AFG^{c}	Clinical evaluation by 2 specialists (4-point scale) (poor, regular, good, very good)	Improvement in patients' scar in all cases (5 cases reported a very good result, and 3 cases a good result)	IV
Han et al., 2008 [33]	RCT	41	12.5	Facial scars	AFG (12) vs AFG + bFGF (29)	N.R.	Satisfaction rate after one injection: 6 patients (AFG) and 24 patients (AFG + bFGF), after two injections: 50% (AFG) and 82.8% (AFG + bFGF)	II

Author	Design	N	FU	Indication	Therapy/ comparison	Methods	Results	OCEBM
Laurent et al., 2006 [34]	PCS	9	15	Cranio-synostosis surgery	AFG	Patient and clinical evaluation of contour restoring in sub-cutaneous fronto-temporal deformation	Good result (patients), moderate result (observer)	IV
Missana et al., 2007 [35]	PCS	74	11.7	Sequelae of conservative breast treatment	AFG[c]	Clinical evaluation of contour restoring by 2 surgeons (3-point scale)	Good to very good result in 64 patients (86.5%) and moderate result in 10 patients (13.5%)	IV
Sterodimas et al., 2011 [36]	RCT	20	18	Congenital or acquired facial tissue defects.	AFG (10) vs ADSC-enriched lipografts (10)	Patient satisfaction of corrected facial defect (5-point scale)	Excellent result in 2 patients (10%), very good result in 16 patients (80%) and good result in 2 patients (10%) (	II

Abbreviations: Significant result; $p < 0.05$ (a) , basic Fibroblast Growth Factor (bFGF), AFG according to Coleman's technique (c), Autologous Fat Grafting (AFG), mean Follow Up in months (FU), Number of patients (N), Not Reported (N.R.), Oxford Centre for Evidence-Based Medicine 2011 (OCEBM), Platelet-rich Plasma (PRP), Prospective Cohort Study (PCS), Randomized Controlled Trial (RCT), Retrospective Cohort Study (RCS), Stromal Vascular cell Fraction (SVF)

Pain

In seven articles regarding 276 patients, the effect of autologous fat grafting on pain was described (Table 4) [26,27,40–44]. After a follow-up of 6 to 13 months, there was a significant reduction of pain in 133 patients treated with autologous fat grafting for postmastectomy pain syndrome [41,42] in 20 patients with painful and retractile scars located throughout the body [26], and in 20 patients with pain after episiotomy and perineal laceration [44]. Baptista et al. [40] described a complete disappearance of pain in three patients, and six patients reported a decrease in pain. Eight patients reported a reduction in use of pain medications, with a followup of 14 months after treatment with autologous fat grafting according to the Coleman technique [40].

Table 4: Studies that report on outcomes using pain (VAS)

Author	Design	N	FU	Indication	Therapy/ comparison	Methods	Results	OCEBM
Baptista et al., 2013 [40]	RCS	11	6	Surgical management of painful scars	AFG[c]	Pain (VAS) Reduction on analgesics intake	3 patients (27%) had complete disappearance of pain (VAS ≤ 1), 6 patients (54.4%) decrease of pain (mean VAS from 8 to 5), 2 patients (18%) had no improvement 8 patients (72.7%) had a reduction in taking painkillers	IV
Caviggioli et al., 2011 [41]	RCS	113	13	Postmastectomy Pain Syndrome	AFG[c] (72) vs no intervention (41)	Pain (VAS)	Decrease in pain[a] (3.23 ± 2.96 vs 1.04 ± 2.71)	II
Klinger et al., 2013 [26]	PCS	20	12	Painful and retractile scars	AFG[c]	Pain (POSAS)	Reduction of pain[a]	III
Maione et al., 2014 [42]	PCS	88	10	Postmastectomy Pain Syndrome	AFG[c] (57) vs control (35)	Pain (VAS)	Decrease in pain[a] (3.1 ± 2.7 vs 0.9 ± 2.6)	III
Mazzola et al., 2013 [43]	PCS	10	21.3	Tracheostomy scar	AFG	N.R.	All patients were relieved from pain	IV
Sardesai et al., 2007 [27]	PCS	14	12	Facial scars	AFG[c]	Pain (POSAS)	Improvement in pain	III
Ulrich et al., 2012 [44]	PCS	20	6	Pain after episiotomy and perineal laceration	AFG[c]	Short-form McGill Pain Questionnaire including the Present Pain Intensity index and VAS	Decrease[a] in McGill Pain questionnaire, Present Pain intensity index[a] and VAS score[a]	III

Abbreviations: Significant result; $p < 0.05$ (a) , AFG according to Coleman's technique (c), Autologous Fat Grafting (AFG), mean Follow Up in months (FU), Number of patients (N), Not Reported (N.R.), Oxford Centre for Evidence-Based Medicine 2011 (OCEBM), Patient and Observer Scar Assessment Scale (POSAS), Prospective Cohort Study (PCS), Randomized Controlled Trial (RCT), Retrospective Cohort Study (RCS), Visual Analogue Scale (VAS)

Itch

Effects of autologous fat grafting for the treatment of itch were described in three studies on 44 patients (Table 5) [26,27,43]. Ten patients were relieved from itch, and 14 patients reported an improvement of itching, with a follow-up ranging from 12 to 21.3 months [27,43]. Klinger et al. [26] reported no reduction of itch after 12 months.

Table 5: Studies that report on outcomes using itch

Author	Design	N	FU	Indication	Therapy/ comparison	Methods	Results	OCEBM
Klinger et al., 2013 [26]	PCS	20	12	Painful and retractile scars	AFG[c]	Itching (POSAS)	No reduction of itching	Ⅲ
Mazzola et al., 2013 [43]	PCS	10	21.3	Tracheostomy scar	AFG	N.R.	All patients were relieved from itching	Ⅳ
Sardesai et al., 2007 [27]	PCS	14	12	Facial scars	AFG[c]	Itching (POSAS)	Improvement of itching	Ⅲ

Abbreviations: Significant result; $p < 0.05$ (a) , AFG according to Coleman's technique (c), Autologous Fat Grafting (AFG), mean Follow Up in months (FU), Number of patients (N), Not Reported (N.R.), Oxford Centre for Evidence-Based Medicine 2011 (OCEBM), Patient and Observer Scar Assessment Scale (POSAS), Prospective Cohort Study (PCS), Randomized Controlled Trial (RCT), Retrospective Cohort Study (RCS)

Autologous Fat Grafting Combined with Other Treatments

In four studies that included 176 patients, autologous fat grafting was combined with other treatments (Table 6) [39,45–47]. In 39 patients treated with autologous fat grafting + 40 percent platelet- rich plasma (29 patients) and autologous fat grafting + 40 percent platelet-rich plasma + insulin (10 patients), an absence of asymmetry of 100 percent and 70 percent was seen, respectively. There was also a 65 percent reduction of deformity and a 30 percent decrease in paresthesia after a follow-up of 12 months [39]. Cervelli et al. [45] reported improvement in texture, color, and scar contour in 20 patients treated with autologous fat grafting according to the Coleman technique + 25 percent platelet-rich plasma + non-ablative laser. An overall clinical assessment by an independent physician showed an improvement of 18 percent in 20 patients treated with autologous fat grafting according to the Coleman technique + 25 percent platelet-rich plasma and 45 percent in 20 patients with autologous fat grafting according to

the Coleman technique + 25 percent platelet-rich plasma + nonablative laser after 6 months. Patient satisfaction rate after 6 months was more than 50 percent in 64 patients if treated with autologous fat grafting according to the Coleman technique + platelet-rich plasma + laser carbon dioxide [46] and 84 percent in 40 patients if treated with autologous fat grafting according to the Coleman technique + 25 percent platelet-rich plasma, whether or not in combination with a nonablative laser [45]. Momoh et al. [47] reported 90.6 percent contour improvement in 33 patients after 6 months with autologous fat grafting according to the Coleman technique combined with a forked cannula.

Table 6: Studies that report on outcomes using AFG combined with other treatments

Author	Design	N	FU	Indication	Therapy/ comparison	Methods	Results	OCEBM
Cervelli et al., 2012 [39]	RCT	39	12	Soft tissue defects and scars	AFG + PRP (29) vs AFG + PRP + insulin (10)	Subjective evaluation of dysesthesia/parasthesia and pain	Decrease in parasthesia of 30% (AFG + 40% PRP + insulin) and 35% (AFG + 40% PRP) No pain reported	II
						Objective evaluation of asymmetry, deformity, irregularity and dyschromia	Absence of asymmetry up to 70% (AFG + 40% PRP + insulin) and 100% (AFG+40% PRP) Reduced percentage of deformity up to 60% (AFG + 40% PRP + insulin) and 70% (AFG + 4-% PRP)	
Cervelli et al., 2011 [45]	RCT	40	6	Traumatic scars	AFG^c + PRP (20) vs AFG^c + PRP + nonablative laser (20)	Patient satisfaction grading the aesthetic and functional quality of the scar (4-point scale)	84% good to excellent improvement, 16% poor to fair improvement	II
						Clinical evaluation by an independant physician according to MSS criteria (4-point scale) to measure scar color, contour, texture and distortion and matte/shiny	Overall clinical assessment: Improvement of 18% with AFG^c + 25% PRP and 45% with AFG^c + 25% PRP + nonablative laser Improvement in texture, colour and scar contour in patients treated with AFG^c + 25% PRP + nonablative laser	
Momoh et al., 2011 [47]	RCS	33	6	Cicatricial Contracture deformities in Breast reconstruction	AFG^c + Forked cannula	Clinical evaluation by the authors	90.6% Contour improvement	IV
Nita et al., 2013 [46]	PCS	64	6	Atrophic (43) and contractile (21) scars	AFG^c + PRP + Laser CO_2	Patient satisfaction rate (4-point scale) based on scar appearance, skin condition, symptoms, edema, ecchymosis, recovery time	Overall satisfaction rate was over 50%	IV

Abbreviations: Significant result; $p < 0.05$ (a) , AFG according to Coleman's technique (c), Autologous Fat Grafting (AFG), mean Follow Up in months (FU), Number of patients (N), Not Reported (N.R.), Oxford Centre for Evidence-Based Medicine 2011 (OCEBM), Platelet-rich Plasma (PRP), Prospective Cohort Study (PCS), Randomized Controlled Trial (RCT), Retrospective Cohort Study (RCS)

Complications

Complications included a superficial abdominal hematoma, which required percutaneous surgical drainage [28]. Four patients developed infection, which was treated successfully with antibiotics [30,31,36]. Two patients developed a reactivation of herpes infection, which resolved in 4 days without leaving pigmented lesions. Postlipofilling edema was reported, lasting up to 1 month [23,45,46]. Four patients had temporary hyperpigmentation, which disappeared within 3 months [45]. Reinjection of autologous fat was reported; 63 patients required a second operation, and 19 patients required a third operation [23,28,29,31–36,43,44,47].

Safety

Delay et al. [31] reported a recurrence of cancer in six patients (14.3 percent) treated with autologous fat grafting for sequelae of conservative treatment after breast cancer, with a mean follow-up of 20.6 months. However, in only one case (2.4 percent) was the ipsilateral side affected. In three patients, the contralateral side was affected, one patient had bone and liver metastases, and one patient had a lymph node metastasis [31]. Amar et al. [37] reported that 66.7 percent of the patients remained category 2 of the American College of Radiology, after a follow-up of 9 months; postoperative results were not reported [37]. An increased incidence of fat necrosis was seen in 7.3 percent to 50 percent [30,35,37].

Discussion

This study provides an overview of the literature regarding autologous fat grafting for the treatment of scar tissue and scar-related conditions. It is remarkable that autologous fat grafting is used as a treatment strategy for the very diverse symptoms of scar tissue. These include the appearance of the scar, skin characteristics, restoration of volume and/or (three-dimensional) contour, pain, and itch. The supposed mechanism of action of the treatment is often unclear. One rationale for using autologous fat grafting for improving scar quality stated that it has a volume-increasing effect and improves skin quality by stimulating the neosynthesis of collagen fibers [12–15]. The mitigating effect of autologous fat grafting on different types of pain was postulated to be a result of changes in the microenvironment and secretion of substances by mesenchymal cells of the graft, which may cause prolonged analgesia [16–18]. In general, positive results

were reported for all of the treatment objectives. However, one should be aware that there is an inherent bias for publication of positive results. Also, the evidence level of the majority of studies was low to moderate, and the quality of the studies with regard to methodology was relatively poor. The level of evidence in most studies was IV [22,23,28–30,32,34,35,37,40,43,46–48], and only four randomized controlled trials were included [33,36,39,45]. The results indicate that autologous fat grafting improves the appearance of the scar and the skin characteristics. The treated areas regain dermal characteristics similar to normal skin, confirmed by both clinical and histological evaluation [22–27]. Resorption of volume after autologous fat grafting is an important outcome and remains a challenge. Undercorrection is the most common complication [59]. Past literature reported an absorption range of 10 percent to 67 percent [60–62]. In this review, similar results are found. Volume maintenance of 31 percent to 90 percent is reported in 104 patients based on preoperative and postoperative photos after a follow-up of 12 to 18 months [25,38,39]. Two articles described volume retention in 25 patients with three-dimensional breast imaging by computed tomography scan after a follow-up of 9 months to 3 years, with an absorption rate ranging from 44 percent to 47.5 percent [30,37]. Autologous fat grafting also seems to significantly decrease patient pain perception [26,41,42,44]. However, it should be considered that the injections alone could have beneficial effects on pain management by loosening the retractile skin. In the articles included in this review, the authors did not compare the experimental results with those of a control group treated with placebo injections only (e.g., with a saline solution). Nor did they describe how many times a patient was injected and thereby releasing possible adhesions. Only Klinger et al. [26] reported that many radiating passages were made in order to distribute fat in different directions to an ideal form of a web. Variable results are described for the treatment of itch. Two articles described an improvement of itch after treatment with autologous fat grafting [27,43], but Klinger et al. [26] reported no reduction of itch after 12 months. The combination of autologous fat grafting with platelet-rich plasma is believed to increase fat grafting survival and function [39,45]. Cervelli et al. [45] reported the most effective scar treatment as autologous fat grafting and platelet-rich plasma combined with nonablative laser resurfacing. The combination of autologous fat grafting, plateletrich plasma, and laser therapy seems to be a safe and effective treatment for scar tissue. However, only two articles that included 84 patients combined these treatments [45,46]. Future studies are needed to confirm these results. The process safety is relevant in this type of treatment, particularly when patients have a history of breast

cancer. The greatest fear is a delayed diagnosis of breast cancer as a result of irregularities and nodules of the injected fat into the breast [30]. Another problem could be development or recurrence of cancer. Several articles demonstrated the process safety [48,63,64]. Delay et al. [48] investigated the safety of fat injection into the breast among 880 procedures, and oncological follow-up showed no increased risk of local recurrence or development of a new cancer. Other studies reported a local recurrence rate of up to 4 percent [63,64]. This is comparable to follow-up studies of locoregional recurrence of breast cancer after breast-conserving therapy, which is up to 3.8 percent [65,66]. Our results show a recurrence of 14.3 percent in 42 patients, but only one (2.4 percent) local recurrence [31]. In the other patients, there were metastases, or the controlateral breast was affected, which could be second primary malignancies. A local recurrence rate of 2.4 percent corresponds to the numbers described in the literature. Hence, there is no indication that autologous fat grafting used to treat scar tissue resulting from breast cancer treatment increases the risk for recurrence of breast cancer. There are several limitations regarding the lack of strong evidence of autologous fat grafting for the treatment of scar tissue. The current available literature includes trials with small sample sizes, absence of control groups, and a relatively short follow-up. In this review, the methodologically less strong trials were also included. All 26 studies showed an improvement of the scar tissue treated with autologous fat grafting. However, only five studies reported statistical analyses, which showed a statistical significant improvement, compared with a control group or baseline [26,27,41,42,44]. The other articles did not report statistical analyses. Therefore, their outcomes should be interpreted with caution. Furthermore, only one of the articles described blinding of the observers [28]; two studies described evaluation by an independent observer [22,45]; and only six studies used objective parameters to assess results [25–27,30,31,37]. Therefore, observer bias could have influenced the results of the studies. Two articles were partially supported by a grant from a transplantation agency of Lazio (Italy), but no conflict of interest is reported [38,39].

Conclusions

This systematic review suggests that autologous fat grafting provides a beneficial effect on scar tissue and scar-related conditions with limited side effects. However, the evidence is still very sparse. A significant improvement of scar appearance, skin characteristics, and in pain is reported in a few studies, with a

follow-up ranging from 6 to 13 months. Itch and restoration of volume and (three-dimensional) contour are also improved. However, the articles included in this review are mostly of low to intermediate evidence level and are lacking in methodological quality. Future randomized controlled trials with a methodologically strong design are necessary to confirm the effects of autologous fat grafting on scar tissue and scar-related conditions.

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Chapter 5

Autologous Fat Grafting: A Promising Technique with Various Indications. Reply: The Use of Autologous Fat Grafting for Treatment of Scar Tissue and Scar Related Conditions: A Systematic Review

Negenborn VL, Groen JW, Smit JM, Niessen FB, Mullender MG. Plastic and Reconstructive Surgery 2016 Dec;138(6):1077e-1078e

Sir

We welcome for the opportunity to discuss the promising effects of autologous fat grafting. Caviggioli et al. added a very interesting study published in 2016 regarding the positive effects of autologous fat grafting on scar tissue and scar-related conditions, such as the treatment of postmastectomy pain syndrome [1]. We congratulate the authors for reporting yet another important chapter in the paradigm shift of autologous fat grafting as not simply a filler of defects. It promises not only improvement of aesthetics but also of functional outcome (e.g., treating Dupuytren contractures) [2,3]. So far, this functional benefit of autologous fat grafting has only been shown in small series and case reports. Several countries, including The Netherlands, have difficulties with reimbursement of the treatment, and these additional indications can shed new light on reimbursement issues by insurance companies. As pointed out by the authors, the technique is promising, with minimal side effects. This great potential is also reflected by the enormous number of articles reporting on the subject, which is increasing each year (Fig. 1) [4].

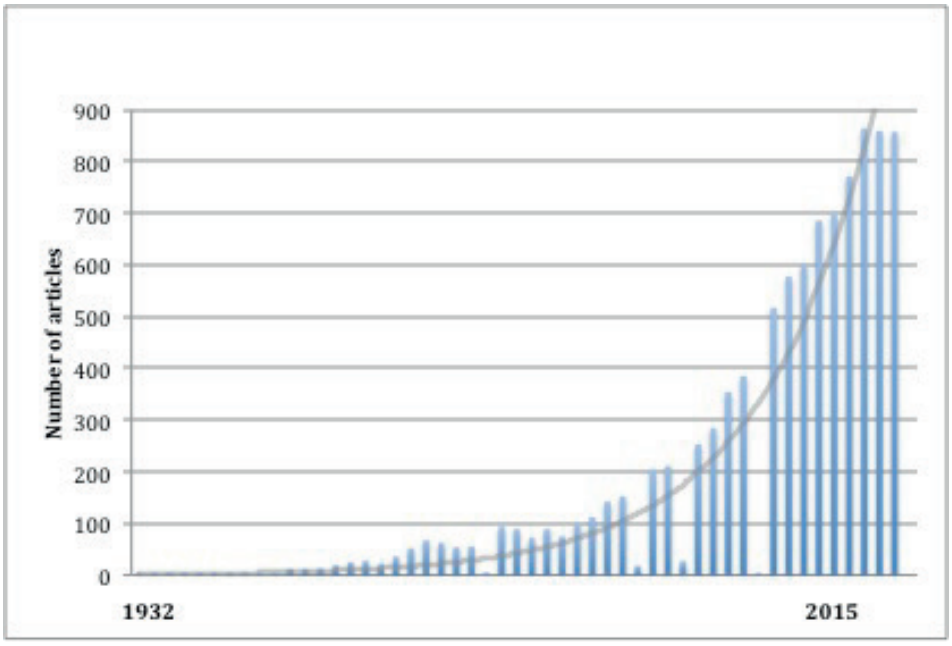

Figure 1. Articles reporting on the use of any form of fat-, lipo- or adipocyte grafting published in Pubmed between 1932-2015

Figure 1: Articles reporting on the use of any form of fat grafting, lipografting, or adipocyte grafting available on PubMed between 1932 and 2015, based on the literature search as reported in the Journal (Negenborn VL, Groen JW, Smit JM, Niessen FB, Mullender MG. The use of autologous fat grafting for treatment of scar tissue and scar-related conditions: A systematic review. Plast Reconstr Surg. 2016;137:31e–43e).

In addition to its analgesic effects, the volume increasing characteristic of autologous fat grafting makes it one of the most preferred reconstructive options. It is autologous and can correct both subtle defects and large volumes. Furthermore, it results in minimal side effects and only few complications. There are several hypotheses about the mechanism(s) by which autologous fat grafting reduces pain sensation, but the exact physiology is still unknown. We suggest that autologous fat grafting decreases the amount of fibrosis by softening or reducing the fibrotic tissue, possibly by revascularization of the scar tissue [5]. We have the impression that this effect occurs over a period of several months. Second, its volume-increasing effect can improve severe skin dimpling and retractions in scar tissue. It is also possible to augment congenital or acquired deformities throughout the entire body surface. The effect of autologous fat grafting is supposed to be stable after 1 year. However, patients still report varying resorption rates after long-term follow-up. Current literature lacks long-term follow-up studies and mainly consists of case series with limited patients included. In the Netherlands, a large multicenter, randomized, controlled trial is currently being conducted comparing external preexpansion and autologous fat transfer versus the conventional tissueexpander/ implant-based breast reconstruction in mastectomy patients (BREAST trial, ClinicalTrials.gov identification number NCT02339779, conducted by Krastev et al. [6]). The patients are measured with a 3D Vectra Imaging System to accurately evaluate volume changes. This randomized controlled trial can confirm the volume gain after autologous fat grafting and will assess whether a higher quality of life is obtained compared with two-stage implant-based breast reconstruction. Secondary outcome measurements are skin elasticity and interstitial pressure to assess the maximal volume to inject. There is still much evidence to be gained regarding the effects of autologous fat grafting, but it may turn out that autologous fat grafting could be rightfully called the liquid gold [7].

Disclosure

The authors have no financial interest to declare in relation to the content of this communication.

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Chapter 6

Oncological Recurrence after Autologous Fat Grafting in Breast Reconstruction; Critical appraisal of the current literature on basic science and clinical studies

Groen JW, Tuinder SMH, Negenborn VL, van der Hulst RRJW. Internal Medicine Review: Vol.3 Issue 2, February, 2017

Abstract

Background: The use of Autologous Fat Grafting (AFG) in surgical procedures of the female breast has gained enormous international interest over the last decade with indications ranging from aesthetic augmentation to the treatment of post-mastectomy-pain-syndromes and breast-reconstructions. One of the most important unanswered questions remains that of oncological-safety, with an almost equal sum of clinical- and basic-science-studies suggesting oncological-safety and an increased risk of oncological recurrence respectively. In this paper the authors aim to provide a comprehensive overview of the overwhelming data currently available on the subject of oncological-safety after AFG for (breast) reconstructive purposes.

Method: An extensive literature search was performed using the following databases; PubMed, Embase.com, Wiley/Cochrane Library and Web of Science. Original studies reporting on AFG for (breast) reconstructive purposes were included and a tabulated overview of data regarding oncological-safety from either a clinical- or basic-science point-of-view are provided.

Results: Thirty-five and twenty-one basic-science- and clinical-studies reported on oncological safety respectively. Thirty-one basic-science-studies described the carcinogenic effects of AFG with most reporting the effects of adipocyte-derived-stemcells in stimulating growth, migration, neo-vascularisation, self-renewal or metastatic-capabilities of different breast-cancer-cell-lines through various pathways. A meta-analysis of clinical-studies on oncological-safety after cancer treatment and breast reconstruction with AFG in a total of 2953 patients reported a locoregional-recurrence-rate of 2.5% and a distant-recurrence-rate of 2.0% with no difference between mastectomy and breast-conserving-therapy patients (p=0.69). However, a significant higher number of locoregional recurrences compared to a control group were found in two sub-cohorts of intra-epithelial neoplasms.

Conclusion: It is clear that more scientific data from both basic science studies using clinical breast cancer samples with representable ASC concentrations as well as clinical studies, preferably RCT's, with a clear distinction between breast cancer types and the recurrence risk after breast-conserving therapy are needed in order to make clear assumptions about oncological safety of AFG.

Glossary of Terms

AFG	Autologous Fat Grafting
ASC	Adipose-derived stem cells
BCCL	Breast Cancer Cell Line
CCL	C-C Motif Ligand
CD	Cluster of Differentiation
c-Met	Hepatocyte Growth Factor Receptor which, in humans is a protein encoded by the MET gene
CXCL	Chemokine (C-X-C Motif) Ligand
CXCR	CXC chemokine receptors
Dkk	Dickkopf (family of proteins with two cysteine rich regions which is involved in embryonic development through its inhibition of the Wnt signaling pathway)
EGFP	Enhanced Green Fluorescent Protein
EMT	Epithelial Mesenchymal Transition
GPI	Glycophosphatidylinosito
HGF	Hepatocyte Growth Factor
HIF	Hypoxia-Inducible Factor
IFN	Interferon
IL	Interleukine
JAK-STAT	Janus kinase (JAK) and two Signal Transducer and Activator of Transcription (STAT) proteins
Matrix Metalloproteinase-9 (MMP-9)	A class of enzymes (matrixin) that belong to the zinc-metalloproteinases family involved in the degradation of the extracellular matrix
MCF-7	Michigan Cancer Foundation-7 (breast cancer cell line)
MDA-MB-231	Cell line of Invasive Ductal Carcinoma
MPE	Metastatic Pleural Effusion
MSC	Mesenchymal Stromal Cells
PBL	Peripheral Blood Lymphocyte
PDGF	Platelet-derived growth factor
RANTES	Regulated on Activation Normal T Cell Expressed and Secreted
SDF	Stromal Cell-Derived Factor
TNF	Tumor Necrosis Factor
WAT	White Adipose Tissue

Introduction

Autologous Fat Grafting (AFG) in surgical procedures of the female breast has evolved from en bloc surgical transplantation of adipose tissue as performed by pioneers like Dr Neuber in 1893 [1] to total banishment in 1987 by the American Society of Plastic Surgeons (ASPS) for concerns of interference with breast cancer surveillance [2]. However, a more recent general acceptance has been reached regarding the procedure, with the implementation of several clinical guidelines [3-5] stating the indications, technique and pitfalls of the procedure. With AFG already accounting for 9.1% of all cosmetic surgical procedures in the world [6] and a tremendous increase of implementation in cosmetic breast augmentation and reconstruction [7], its potential and appeal to both physicians and patients is undeniable. For patients most of the attraction of the procedure arises from the desire to redistribute fat from places were its generally undesired to places where it is not. For physicians AFG offers a relatively easy to perform technique without the need for microsurgical expertise, which can be performed in an outpatient setting with minimal donor site morbidity. In breast cancer patients, AFG has become a vital part of the onco-plastic reconstruction [8] with options ranging from improving the aesthetic results of implant or myocutaneous flap reconstruction by filling folds and adjusting the volume to actually reducing (implant) capsular contracture and pain in post mastectomy pain syndrome [9,10]. With the use of new found techniques like the Breast Enhancement and Shaping System (BRAVA) mega volume breast reconstructions using only AFG have recently been performed [11,12]. While some of the concerns about AFG regarding the interference with breast cancer surveillance in these patients has been subsided thanks to the current radiographic technology in distinguishing normal post-AFG appearances from malignant lesions [13], the debate about the possible oncogenic potency of AFG is at an all-time high.

In 2014 Smit et al. presented a case of a 44 year old woman with recurrent invasive ductal carcinoma 10 months after the last of two AFG sessions for pain and tightness of bilateral mastectomy scars [14]. While numerous cases like this have been described [15-17], clinical studies and systematic reviews have not been able to show a significant increased oncological recurrence after AFG in breast reconstruction [18-22]. In our recently published systematic review [23], meta-analysis of a total cohort of 3020 patients (21 studies) after cancer treatment and breast reconstruction with AFG showed no significant increased oncological locoregional or distant recurrence rates compared to patients without AFG after breast

cancer surgery [24-27]. However, despite a large sum of clinical studies suggesting no significant increased recurrence risk, one of the biggest controversies lays in the fact that basic science in-vivo and in-vitro studies did suggest that there is indeed a significant risk at oncological recurrence when transplanting fattish tissue to previously cancerous environments.

In this paper the authors aim to provide a comprehensive overview of the overwhelming data currently available on the subject of oncological safety after AFG for (breast) reconstructive purposes. A differentiation will be made between basic science and clinical studies as well as studies rejecting or accepting the hypothesis of AFG increasing the chance of oncological recurrence after oncoplastic breast reconstruction.

Method

An extensive literature search was performed, using an updated version of the database created for the purpose of two previously published systematic reviews [23,28]. The following databases were searched from inception between January 2005 and July 2016; PubMed, Embase.com, Wiley/Cochrane Library and Web of Science. The following terms were used (including synonyms and closely related words) as index terms or free-text words: 'fat' or 'adipocyte' or 'lipo' and 'grafting' or 'filling' or 'transplant'. The full search strategies for all the databases can be found in the Supplementary Information of the previously published systematic reviews [23,28]. Original, basic science studies describing the interaction between a component of rëinjected adipose tissue (i.e. adipose-derived stem cells, adipocytes etc) and the recipient site ((dormant) breast cancer cells, stromal cells etc) using in-vitro and/ or in-vivo laboratory models, were found eligible for proofreading. A comparison is made with the clinical oncological recurrence rate as reported by case series, cohort studies and randomized clinical trials on AFG for breast reconstruction purposes, with a mean follow-up of 12 months, reporting on 10 patients or more.

Results

Basic science studies

Thirty-five basic science studies reported on the possible carcinogenic effects of AFG [29-63] with four studies describing the possible tumor-supressing abilities [64-67]. It is beyond the scope of this article to give a full-depth analysis of the different biological pathways through which the different ASC associated factors influence the various BCCL's studied. For this we refer the reader to the relevant articles. Five studies described the carcinogenic effects of ASC's in stimulating growth, migration, neo-vascularisation, self-renewal or metastatic capabilities of different BCCL's when co-cultured using in-vivo and in-vitro techniques [29,30,41-43]. This was instigated through various specific ASC-tumor-cell relations as follows: 1) the HGF/c-Met crosstalk between ASC's and c-MET-expressing breast cancer cells [29], 2) the induced expression of mesenchymal markers on breast cancer cells through ASC mediated tumor micro-environment EMT [42] or 3) through increasing proliferation of several BCCL's directly by ASC secreted Cytokines, Chemokines and growth factors [43]. In-vivo characteristics of these ASC-BCCL cultures when implemented in a mouse model was more specifically reported in five studies [29,31-33,41] with bot Rowan et al. [31] and Ke et al. [32] reporting an increase in metastasis and tumor vascularisation in respectively MDA-MB-231- (triple negative) and 4T1-BCCL's. Two studies [34,35] reported on the effect of adipocyte progenitor cells when injected into a breast tumor, both in the absence as well as presence of ASC's and found that in both cases tumor vascularization increased while only the latter showed additional enhanced local tumor growth. More general tumor promoting capabilities of ASC's were reported in a total of 13 studies [36-40,54-57,60-63]. These featured: 1) ASC migrating capabilities towards tumor sites mediated by PDGF-BB [36] and 2) ASC derived myofibroblasts and cell surface markers such as CD44 contributing to tumor growth, infiltration and metastasis through what is described as the desmoplastic reaction (reaction of stroma to tumor cell-infiltration) [37-39]. Furthermore, the study of Rubio et al. [63] suggested that ASC's themselves when cultured beyond 5 months started to express characteristics of tumor cells, such as chromosome instability and two other studies [60,61] reported that AFG-induced-hypoxia can stimulate breast cancer invasion and metastasis through Hypoxia-Inducible Factor 1-alpha (HIF-1α). Furthermore, when discussing migration there is growing evidence that breast cancer cells can, in fact, be attracted towards ASC's through several chemokine

mediated receptors such as CXCR4, CCL2 and CCL5 [45,46,48-53]. Finally, two studies [58,59] reported on immunomodulatory capabilities of ASC's in favor of tumor growth, expansion and metastasis. Herein, ASC's isolated from the micro-environment of stage III breast cancer showed an increased percentage of CD4+CD25highFoxp3+ T regulatory cells in peripheral blood lymphocytes (PBLs) which suppressed the proliferation of infiltrating CD8+ T cells which are a main component of the autologous antitumor immune response.

Interestingly, in the study of Ryu et al. [65], ASC's have also been reported to affect the immune system by increasing its tumerosuppressant capabilities. In their in-vitro study they reported that high density cultured ASC's express, amongst others IFN- β which suppresses growth of the MCF-7 BCCL besides inducing apoptosis. Furthermore, MCF-7 BCCL was further inhibited through competitive down-regulation of Dkk-1 (dickkopf-1) via the Wnt pathway in the studies of Qiao et al. [64], and Zhu et al. [67].

Table 1: Basic Science; Overview of the current evidence of AFG in Breast Reconstruction and Oncological Recurrence

Author	Year	Outcome of conclusion
AFG as possible carcinogenic factor / stimulant		
Eterno[29]	2014	Adipose-derived stem cells (ASCs) in fat tissue can (in-vitro/ -vivo) exacerbate tumorigenic behavior of c-Met-expressing breast cancer cells through HGF/c-Met crosstalk between ASCs and breast cancer cells; enhancing tumor cells migration, acquiring a metastatic signature, and sustaining tumor self-renewal.
Kuhbier[30]	2014	In co-cultures, T47D breast carcinoma cells and adipose-derived stem cells displayed a change towards a more malignant phenotype associated with higher rates of metastasis and worsened prognosis as caused by direct intercellular contact.
Rowan[31]	2014	Female human donor MDA-MB-231 (triple negative) breast cancer cells were indirectly co-cultured in a conditioned medium with ASCs derived from three healthy female donors. In two cases this stimulated metastasis in a mouse model and in one case this exhibited partial Epithelial Mesenchymal Transition (EMT), expression of Matrix Metalloproteinase- 9 (MMP-9), and increased angiogenesis.
Ke[32]	2013	Mesenchymal Stromal Cells (MSCs) like ASCs increased the proliferation, migration, and efficiency of mammosphere formation of 4T1 breast cancer cells in an in-vivo mouse model. When co-injected into the mouse mammary fat pad, enhanced tumor growth and increased spontaneous lung metastasis was found. Moreover, longitudinal fluorescence imaging of tumorigenesis revealed that MSCs created a vascularized environment which enhances the ability of 4T1 cells to colonize and proliferate.
Zimmerlin[33]	2011	ASCs enhanced the proliferation of Metastatic Pleural Effusion (MPE) cells when co-cultured. Furthermore, in xenografts experiments, active CD90+ MPE cells were tumorigenic when co-injected with ASCs.
Orecchioni[34]/ Bertolini[35]	2013	Progenitor cells isolated from human adipose tissue generated mature endothelial cells and capillaries within a breast tumor but their cancer-promoting effect in the breast was limited. However, in the presence of ASCs, new vessel formation was accompanied by enhanced local tumor growth.
Gehmert[36]	2010	ASCs have migrating capabilities towards tumor sites. A feature partly mediated by recombinant PDGF-BB.
Chandler[37] / Bochet[38]	2012/ 2013	Invasive breast cancer cells present myofibroblasts – partly derived from neighbouring ASCs - in the stromal compartment. There is evidence that both ASCs and myofibroblasts contribute to the desmoplastic reaction (reaction of stroma to tumor cell-infiltration), which facilitates tumor growth, infiltration and metastasis.
Hass[39]	2012	ASCs surface markers like CD44 are capable of attaching MMP's which in turn can characterize the desmoplastic reaction by increasing the extracellular matrix deposition and vascularization of the extracellular matrix.
Zhang[40]	2012	There is in vivo evidence that ASCs can travel from white adipose tissue (WAT) to breast cancer tumors in mouse models. Simultaneously breast cancer cells can independently induce an increase in systemic ASC rate. Elevated levels of ASCs can be recruited by tumors, incorporated in blood vessels as pericytes and differentiate into adipocytes. Subsequently, increased tumor vascularisation was found to be associated with these elevated intra-tumoral adipocytes.
Mandel[41]	2013	Human MSCs (like ASCs) caused an in-vitro growth stimulation of MDA-MB-231 breast cancer cells, when co-cultured. Furthermore, through direct cellular interactions, MSC also induced expression of the GPI-anchored CD90 molecules in breast cancer cells. In-vivo this caused increased tumor size, elevated neo-vascularization and enhanced metastatic capacity in a mouse model.

Author	Year	Outcome of conclusion
Devarajan[42]	2012	In-vitro/ -vivo analyses of ASCs conditioned mediums showed an induced expression of mesenchymal markers by (4T1) breast cancer cells as well as anchorage-independent growth of breast cancer cells. This was thought to be the result of ASCs interaction with the cancer microenvironment via platelet-derived growth factor-D (PDGF-D) by inducing EMT in a paracrine fashion.
Kucerova[43]	2011	ASCs (or adipose tissue-derived human mesenchymal stromal cells) increased proliferation of MDA-MB-361, T47D and EGFP-MCF7 BCCL's as a results of ASCs secretion of cytokines, chemokines and growth factors.
Burger[44] / Kang[45] / Krohn[46] / Rhodes[47,48]	2006/ 2005/ 2009/ 2010/ 2011	ASCs secrete chemokine stromal cell-derived factor-1 (SDF-1/CXCL12) which can attract cancer cells through its cognate receptor CXCR4. CXCR4 stimulates metastasis, survival and growth of neoplastic cells in a paracrine fashion and angiogenesis by attracting endothelial cells.
Lu[49] / Yoshimura[50]	2009/ 2013	In-vitro/ -vivo analysis of overexpression of the chemokine (C-C motif) ligand 2 (CCL2) by ASCs promotes metastasis to bone and lung of MDA-MB-231 human breast cancer cells. This process was mediated through tumor-associated macrophages recruited by the CCR2 chemokine receptor.
Yaal-Hahoshen[51] / Pinilla[52] / Svensson[53]	2006/ /2009/ 2015	The chemokine RANTES (or Regulated on Activation Normal T Cell Expressed and Secreted) also known as CCL5, is produced in higher levels by ASCs than other stem cells and is associated with increased breast cancer cell motility and metastatic capabilities in in-vivo studies.
Kamat[54] / Welte[55] / Ritter[56] / Zimmerlin[57] Walter[62]	2015/ 2013	The following additional ASCs derived factors have been correlated to invasiveness and migratory potential of breast cancer cells: TNF-α, eotaxin, IL-6, IL-8, IL-10
Razmkhah[58] / Yang[59]	2011/ 2007	In-vitro analysis of ASCs isolated from stage III breast cancer tumors showed an increased percentage of CD4+CD25highFoxp3+ T regulatory cells in peripheral blood lymphocytes (PBLs) cultured with the supernatant of the same tumor. CD4+CD25highFoxp3+ T regulatory cells have immune-regulatory effects and have been shown to suppress the proliferation of autologous infiltrating CD8+ T cells, thereby providing a immunosuppressant network that allows tumor cells to grow, expand and metastasize.
Wang[60]/ Nalwoga[61]	2014/ 2016	The local hypoxia that AFG is thought to produce in the recipient milieu induces expression of Hypoxia-Inducible Factor 1-alpha (HIF-1α) which can potentially stimulate breast cancer invasion and metastasis.
Rubio[63]	2005	In-vitro analysis of isolated ASCs cultured beyond the expansion period of 6-8 weeks for up to 4-5 months showed spontaneous transformation (chromosome instability, altered phenotype) attaining them characteristics of tumor cells.
AFG as tumor-suppressor		
Ryu[65]	2014	In-vitro analysis of ASCs cultured at high density express Interferons (IFNs) that regulate cellular and immune responses as well as antiviral and anti-tumor activity. IFN-β suppresses the growth of MCF-7 human breast cancer cells and induces apoptosis through JAK-STAT1 intracellular signaling pathways. In preliminary results, this cytotoxic activity was also shown from ASC conditioned medium on the MDA-MB-231 BCCL.
Qiao[64]/ Zhu[67]	2008/ 2009	In-vitro/ -vivo analysis of human MSCs showed an inhibitory effect on MCF-7 breast cancer cells through competitive down-regulation of Dkk-1 (dickkopf-1) via the Wnt pathway
Sun[66]	2009	MSCs isolated from both human umbilical cord and adipose tissue reduced growth and lung-metastasis of human breast cancer cells in a mouse cancer metastasis model.

Clinical Studies

For a complete overview of the oncological outcomes of the clinical studies we refer to our recently published systematic review [23]. Herein twenty-one studies reported on oncological safety after cancer treatment and breast reconstruction with AFG in a total of 3020 patients. There were 1371 and 512 cases of invasive carcinomas and carcinomas in situ respectively. One study [68] was excluded from the meta-analysis due to aberrant formulation of the definition of recurrence. Meta-analysis over the total cohort of the remaining twenty studies (n= 2953) showed an LRR of 2.5% (95% confidence interval (CI) 1.7-3.7) and a DR of 2.0% (95% CI 1.1-3.5) with no difference between MST and breast-conserving therapy (BCT) patients (p=0.69). The local and distant recurrence rates in this study were lower than those of patients who underwent MST with immediate breast reconstruction (LRR: 2.5% vs. 5.2%; DR: 2.0% vs. 13.9%) [26] and after BCT with whole-breast irradiation (LRR 2.4% and DR 8.0%) [69]. Two sub cohorts of intra-epithelial neoplasia patients receiving breast reconstruction with AFG were studied by Petit et al. [16,17]. Herein they found a significant higher number of locoregional recurrences compared to a control group in 37 and 59 patients (p= <0.0001 and p= 0.02) respectively.

Table 2: Clinical Evidence; Overview of the current evidence of AFG in Breast Reconstruction and Oncological Recurrence

Clinical Studies showing no increased oncological recurrence rate after breast reconstruction with AFG:

Author	Year	Study population characteristics							Relevant Outcomes		Level of Evidence
		# pat.	Mean age	Initial surgery	Invasive carcinomas	CiS	RT	Mean follow up (months)	Locoregional recurrence	Distant recurrence	
Pierrefeu-Lagrange[70]	2006	30	51	NR	NR	NR	NR	12	0/30	0/30	IV
Delay[71]	2008	42	50.7	BCT	39/42	3/42	36/42	31.2	1/42	5/42	IV
Delay[72]	2009	734		NR	NR	NR	NR	-120	0/734	0/734	IV
Rigotti[73]	2010	137	/	mMST	105/137	31/137	22/137	76.8	5/137	0/137	IV
Rietjens [74]	2011	158/ 191b	48	MST 114/191, BCT 77/191	NR	NR	96/158	18.3	1/158	0/158	IV
Petit[75]	2011	513	52.1	MST 370/513, BCT 143/513	405/513	108/513 (101 duct, 7 lob)	395/513	19.2	13/513	16/513	IV
Sarfati[76]	2011	28	45	MST 28/28	28/28	0/28	28/28	17	0/28	0/28[a]	IV
Petit[16]	2012	321	/	MST 196/321, BCT 125/321	284/321	37/321, DIN 35/321 LIN 2/321	NR	45.3	8/321	5/321	IV
Perez-Cano[68]	2012	67	52	BCT 67	NR	NR	61	12	0/67	NR[b]	II
Constantini[77]	2012	22	50.8	MST 14/22, BCT 8/22	NR	NR	15/22	12	1/22	0/22	IV
Seth[78]	2012	69/ 90b	49.4	MST 69/69	50/90[c]	17/90	prO 2/90 poO 74/90	24.8	0/69	0/69	IV
Sarfati[79]	2013	68	46	MST 68/68	NR	NR	68/68	23	0/68	3/68[d]	IV
Doren[80]	2012	278[e] 448b	51	MST 278/278	94/223	129/223	prO 56/278 poO 34/278	56.2	6/278	3/278[f]	IV
Riggio[81]	2013	60	49	MST 60/60	53/60	5/60[g]	NR	90 (median)	2/60	5/60	III
Ihrai[82]	2013	64	/	MST 50/64, BCT 14/64	51/60	10/61[h]	NR	46	2/64	3/64	IV

Clinical Studies showing no increased oncological recurrence rate after breast reconstruction with AFG:

Author	Year	Study population characteristics						Relevant Outcomes		Level of Evidence
		# pat.	Mean age	Initial surgery	Invasive carcinomas	CiS	RT	Mean follow up (months)	Locoregional recurrence	Distant recurrence	
Longo[83]	2014	21	36.64 38.70	nsMST 21/21	NR	NR	10/21	34.8 17.2	0/21	0/21	III
Kim[84]	2014	102	46.3	MST 102/102	60/102	42/102	NR	28.7	1/102	0/102	IV
Semprini[85]	2014	151	/	BCT 151/151	115/151	25/151[i]	151/151	45	0/151	0/151	IV
Molto-Garcia[86]	2014	37	55	BCT 37/37	35/37	2/37	NR	12	0/37	0/37	IV
Brenelli[87]	2014	59	50	BCT 59/59	52/59	7/59	56/59	34.4	3/59	1/59	III

Clinical Studies showing a significant increased oncological recurrence rate after breast reconstruction with AFG:

Author	Year	Study population characteristics			Relevant outcomes		p-value
		# pat.	Type of Carcinoma	Follow-Up	Locoregional recurrence	Matched control group	
Petit[16]	2012	37	Carcinoma in Situ: 37/321 Ductal Intra-epithelial Neoplasa 35/321 Lobular Intra-epithelial Neoplasa 2/321	45.3 (total cohort)	4/37	0/74	< 0.0001
Petit[17]	2013	59	Carcinoma in Situ: 59 Ductal Intra-epithelial Neoplasa 57/59 Lobular Intra-epithelial Neoplasa 7/59	38 (median)	6/59	3/118	0.02

Abbreviations : Breast Conserving Therapy (BCT), Mastectomy (MST), Radiotherapy (RT), Preoperative (prO), Postoperative (poO), Carcinoma in situ (CiS), Ductal Intra-epithelial Neoplasia (DIN), Lobular Intra-epithelial Neoplasia (LIN), ductal (duct), lobular (lob), Not Reported (NR), nipple-sparing (ns),

Superscripts:

[a]: There was one case of contralateral breast cancer.

[b]: A patient's bone metastasis was considered a natural progression of the disease.

[c]: Twenty-two reconstructions were prophylactic or histological data showed no tumour.

[d]: There were two cases of carcinoma of the contralateral breast, of which one showed simultaneous metastatic bone and liver disease.

[e]: Of the 278 patients receiving mastectomies, 34 were prophylactic and data is missing from 21 patients.

[f]: One patient died of disease, without specification.
[g]: There was one additional patient with a medullary carcinoma and one with Paget's disease.
[h]: Three patients had Phyllodes.
[i]: Missing data: 11/151

Discussion

Summary

The last two decades AFG, as part of reconstruction of the female breast after cancer surgery, has evolved from a general reluctance to an overall acceptance due to the simplicity and minimal invasive character of the technique as well as the good aesthetic results achieved. The question regarding the oncological safety of the technique is still largely unanswered, much debated and remains the topic of a growing number of publications in both basic science and clinical studies. However, with this expanding number of studies, a definitive answer only seems farther away, mainly because of the discrepancies between basic science [29-63] and clinical studies [16,17,68,70-87]. Most basic science studies (table 1) focus on the paracrine, exocrine/ endocrine and autocrine secretions of adipose-derived stem cells (ASCs) and their pro-tumorigenic effects on the recipient site as well as on possible remaining dormant breast cancer cells, especially in case of breast conserving therapy (BCT). Several in-vitro studies [29,41,42] showed an increased growth and metastatic profile of different BCCL's when co-cultured with ASCs. A finding that was substantiated by in-vivo [29,32] proven increased tumor size and frequency of metastatic disease. Furthermore, there is evidence that ASCs have the ability to migrate towards tumor sites [36,40] and breast cancer cells, in turn, can increase the number of ASCs [40]. Besides this synergistic loop between ASCs and breast cancer cells there is also evidence of pro-tumorigenic, immune-modulatory ASC capabilities with increased numbers of T-regulatory cells which can supress CD8+ T-cells, thereby allowing increased tumor cell growth, expansion and metastasis [58,59]. What is interesting is that ASCs also have cytotoxic immune-modulatory effects on breast cancer cells, through the expression of Interferon's by supressing growth and inducing breast cancer cell apoptosis [65]. Until now, most clinical studies [68,70-87] (table 2) have not been able to show a significant increased risk of oncological recurrence, whether locoregional (LR) or distant metastasis (DM), after AFG. In a recently published systematic review on clinical studies [23], we found a LR of 2.5% with a DM of 2.0% with no difference between MST or BCT surgery which are in line with the LR rate (2.2%) in breast cancer patients after AFG as reported in the systematic review by Charvet et al. [88]. However, in the 2012 retrospective matched cohort study of Petit et al., a disproportionate number of LR were found in a subset of 37 out of 321 patients with intraepithelial neoplasms (35 DIN vs 2 LIN) receiving

AFG after MST or BCT compared to 74 matched cohort patients (4/73 vs 0/74, P=<0.0001) [16]. The same findings were reported by the authors a year later when they found a 18% 5-year cumulative risk in patients receiving AFG after BCT or MST for intraepithelial neoplasms compared to 3% in a matched control group (P=0.02) [17].

Critical appraisal of the current literature

Some of the discrepancies between basic science studies and the results of the clinical reports may be explained by several limitations of both studies. Basic science studies, for example, often use banked BCCL's which may not adequately resemble in-patient tumor biology and are often more durable and mutated. On the other hand, the concentrations of ASCs cultured with these BCCL's in in-vivo and in-vitro studies, are much higher than that reported in both the average lipoaspirate ($4.0x10^5 \pm 2.0x10^5$ ASCs per ml of lipoaspirate) [89,90] as well as ASCs enriched fat grafts [91,92]. Thus, narrowing the gap between basic science and clinical studies can be partly achieved by using clinical breast cancer samples and more representable concentrations of ASCs, preferably from the same patient. Clinical studies are currently limited by a sheer number of methodological factors such as the generally retrospective design lacking a matched control group, the heterogeneity between patients and types of breast cancer (receptor status, invasive vs intraepithelial, staging) as well as the short follow up periods on oncological recurrence. In regard to control group matching, the recently published study by Gale et al. [93], is worth mentioning. In a 1:2 control matched study of 328 woman with previously treated malignant breast disease who underwent AFG no significant excess of local, regional or distant recurrences were found, after a mean follow up of 32 months post-AFG. Interestingly, there were no recurrences in the subset of 27 patients with ductal carcinoma in situ which the authors attributed to the long disease-free interval (54 months) between primary oncologic surgery and fat grafting. Even though no definitive conclusions can be drawn from this small sample size, a recommendation for clinical practice can be to wait out this period of 50+ months, before commencing AFG. Krumboeck et al., recommended waiting 5 years after BCT, based on a general 9% recurrence peak reported in this timeframe but further clinical studies are needed to verify these findings after AFG [21,94]. Another limitation of the current published clinical series (both pro- and retrospective), is the absence of methodology that adequately adjusts and/ or stratifies for patho-biological charac-

teristics of the tumor, such as (molecular) subtype, size and risk-profile. Further clarification of both these clinical factors (cancer cell residuality after BCT and –subtype) is of great importance since the possibility of residual (dormant) breast cancer cells after BCT only partly explains the mechanism why LR or DM can occur after AFG. The when, how and to what extent, might depend much more on these patho-biological characteristics of the tumor and results concerning recurrence risk obtained in one subtype cannot be extrapolated to another. The importance of this concept was recently shown by the preliminary results presented by Fertsch et al., during the European Breast Cancer Conference 2016 [95]. In a subgroup analysis of 100 matched patients undergoing AFG after Deep Inferior Epigastric Perforator Flap (DIEP), with mean follow up of 31 months, they found that AFG increased the recurrence risk in patients with HER2-positive breast cancer, high-grade G3 neoplasia and patients with nodal involvement.

It is clear that more scientific data from both basic science studies using clinical breast cancer samples with representable ASC concentrations as well as clinical studies, preferably RCT's, with a clear distinction between breast cancer types and the recurrence risk after BCT are needed. In the Netherlands a large multicentre RCT is currently being conducted comparing external pre-expansion and AFG versus the conventional tissue-expander/implant based breast reconstruction in mastectomy patients (BREAST trial, registered at ClinicalTrials.gov: NCT02339779, T. Krastev et al.) with oncological events as one of the secondary outcomes [96].

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Chapter 7

European Survey Study amongst Plastic/ Breast Surgeons on the use of/ and opinion towards Autologous Fat Transfer; with emphasis on Breast Surgery

Groen JW, Piatkowski AA, Sawor JH, Wilschut JA, Ritt MJPF, van der Hulst RRJW. Submitted

Abstract

Background: The popularity of Autologous-Fat-Transfer causes an up-rise in sophisticated scientific research and clinical implementation. While results from the former are well-documented, important aspects of the latter are less recognized. The aim of this study is to ventilate the experience of European surgeons and highlight differences between countries and level of experience.

Method: An international survey-study about surgeon background, besides AFT-familiarity, -technique and –opinion was distributed amongst surgeons from 10 European countries. Differences between countries and level of experience were analyzed using a logistic-regression model.

Results: The mean respondent age, out of 358 completed questionnaires, was 46 years. Ninety-seven percent of respondents were plastic surgeons, who practiced AFT mostly in breast-surgery and considered themselves experienced with the technique. The thigh and abdomen were less favored harvest-locations by the Belgium and French respondents respectively and both the French and Austrian respondents preferred manual-aspiration over liposuction in harvesting the fat. Despite minor differences between countries and experience the intra-glandular space was injected in all subgroups.

Discussion: Despite an obvious adherence to Coleman's Method in Europe, deviations thereof become more apparent. While this may offer opportunities in finding the golden standard in AFT, unsafe practice like intra-glandular AFT should be avoided until scientific clarification regarding oncological safety.

Conclusion: The expanding use of AFT in Europe will lead to more experience and heterogeneity regarding the technique. Guidelines aid clinical practice and cause reproducibility but adherence regarding important aspects like injection-planes deviate into possible risky territory. Therefore, European surgeon education should focus on these issues.

Introduction

Autologous Fat Transfer (AFT) is becoming an increasingly popular procedure in various areas of plastic surgery. Whether used as permanent filler in facial rejuvenation [1,2] or as a volume enhancing technique in addition to onco-plastic or cosmetic surgery of the breast, much is written regarding efficacy and safety as well as various techniques and satisfaction [3-5]. Thus, popularity and acceptance is growing. Vice-versa, this acceptance leads to more and better research currently being conducted [6]. Regarding the AFT-technique, the systematic review of Strong et al. [7] recently showed higher retention rates in clinical studies with centrifugation - as opposed to sedimentation – and slow reinjection into less mobile areas. However, this same advantage could not be found in experimental animal and in vitro studies. Satisfaction rates among patients and surgeons are generally assessed with the use of likert-scales [8-11] or validated questionnaires like the breast-Q [12]. Despite the advantages and rising confidence with the procedure, concerns about oncological safety remain, since several experimental studies show potential danger of interaction between adipose-derived-stem-cells (ADSC) and mammary epithelial cells as well as the potential of CD34+ progenitors in white adipose tissue to promote cancer progression [13-15]. Regardless of the increase in clinical acceptance of AFT, questions regarding the golden-standard in AFT-technique and oncological safety remain, partly because of the gap between clinical- and basic science studies. One way to narrow the gap between the laboratory and clinical practice is by way of professional survey studies. Two survey studies amongst professionals are worth mentioning. Kaufman et al. [16] in 2007 and Skillman et al. [17] in 2013 both performed a national survey concerning the use of AFT amongst 508 US-, and 228 UK plastic surgeons respectively. The former study reported mainly on the use of AFT in facial recontouring, the latter mainly on the use in breasts, but both studies reported a general approval of the technique by surgeons as well as a high rate of surgeon perceived patient satisfaction. The AFT-technique used by the respondents - as reported in the study by Kaufman et al. – rarely deviated from the methods discussed in the literature. Since this study dates from 2007 and reports on US respondents only and given the recent developments in this field [18-20], it is interesting to look at the current situation in Europe. The primary aim of this study is to report on the experience, practice and opinion of plastic surgeons and breast surgeons in Europe with the AFT procedure in general and with special emphasis on breast-surgery. The secondary aim is to highlight the possible differences

between surgeons from the individual participating European countries in terms of AFT-technique, surgeon experience, opinion and expectations.

Methods

An international, multicenter, cross-sectional, closed-ended format, study specific questionnaire was created regarding AFT in general and with emphasis on breast-surgery. The national plastic surgery associations of ten European countries (Netherlands, Belgium, Germany, Great-Britain, France, Spain, Italy, Greece, Austria, and Switzerland) were contacted through email and, after introduction, asked for their participation in distributing this questionnaire amongst their members (active participation). When no reply was received, the organization was contacted on two additional occasions with a minimum of a 2 week interval by telephone during which the method and purpose of the study was explained and the organization was again asked for their participation in the study. Participating organizations distributed the questionnaire amongst its members with a reminder email following after 2-4 weeks. When no active participation could be achieved the email addresses of the members of an organization were actively searched and collected by the first author (JG) through the organizations official websites (passive participation). The questionnaire was constructed in SurveyGizmo, an online digital survey tool and translated in the following languages; Dutch, German, Spanish, Italian and French by either a native speaking colleague or an internet-based translational service (www.onehourtranslation.com). The survey encompassed 36 multiple-choice questions, concerning 4 aspects of AFT, namely; Background, AFT familiarity, AFT-technique and AFT opinion (see figure 1). A free text section was provided at the bottom of the appropriate questions to allow respondents to add personal comments. The completion of the questionnaire was strictly voluntary and without compensation. The completed questionnaires were entered into a database (SPSS Inc., Chicago, IL, USA) by one investigator (JG) for further analysis.

Figure 1: Survey Questions

1) In which country do you currently practice?	( ) Finland
	( ) Greece
( ) The Netherlands	( ) Hungary
( ) Belgium	( ) Israel
( ) Germany	( ) Poland
( ) Great-Britain	( ) Portugal
( ) France	( ) Romania
( ) Spain	( ) Sweden
( ) Italy	( ) Turkey
( ) Austria	( ) Norway
( ) Switzerland	( ) Other - Write In (Required)
( ) Croatia	
2) What is your age?	
3) What is your specialty?	( ) Breast Surgeon
	( ) Other - Write In (Required)
( ) Plastic Surgeon	
4) What is your current position?	( ) Registered medical specialist
	( ) Other - Write In (Required)
( ) Resident	
5) What is your current year of residency?	( ) 3rd
	( ) 4th
( ) 1st	( ) 5th
( ) 2nd	( ) 6th
6) What is your work experience (years since registered medical specialist)?	( ) 5-10 yrs
	( ) 10-15 yrs
	( ) 15-20 yrs
( ) < 5 yrs	( ) > 20 yrs
7) Do you-, or have you ever practiced AFG in general or in surgical procedures to the breast?	( ) I (have) also practice(d) AFG in surgical procedures to the breast
	( ) I have never practiced AFG for any indication
( ) I (have) practice(d) AFG in general (i.e. for other indications then breast surgery)	
8) How many fat transfer procedures do you perform per year?	( ) 10-30
	( ) 30-50
	( ) >50
( ) < 10	
9) Do you perform fat transfer procedures yourself or with a colleague?	( ) With a senior colleague
	( ) With a resident
( ) With a colleague	( ) Myself

10) How experienced do you consider yourself with fat transfer procedures?	( ) Moderately experienced
	( ) Moderately unexperienced
	( ) Unexperienced
( ) Experienced	
11) What is/are the preferred harvest location/donor site(s) for injectable fat? (Mark all that apply)	( ) Thigh
	( ) Flank
	( ) Abdomen
( ) Gluteal	( ) Knee
	( ) Other - Write In (Required)
12) What local anesthesia is used for the donor site?	( ) Wetting solution (50 ml of 1% lidocaine plus 1 ml of epinephrine 1:1000 plus 1 liter of normal saline)
( ) 0.5% lidocaine with epinephrine	( ) Epinephrine alone
( ) 1% lidocaine with epinephrine	( ) Other
13) How much local anesthesia (cc) is used for the donor site?	_____Flank
	_____Abdomen
_____Gluteal	_____Knee
_____Thigh	_____Other
14) What harvest technique is used?	( ) Microcannula according to Coleman technique
	( ) Syringe plus large-bore needle
( ) Liposuction cannula with constant suction	( ) Other:
15) If a liposuction cannula is used, what size is used most commonly?	( ) 3 mm
	( ) 4 mm
	( ) Unknown
( ) 1 mm	( ) Other - Write In (Required)
( ) 2 mm	
16) If a syringe and cannula are used, what size is used most commonly?	( ) 16 gauge
	( ) 18 gauge
	( ) Unknown
( ) 14 gauge	( ) Other - Write In (Required)
17) What kind of fat preparation do you use?	( ) Centrifugation
	( ) Adding Insuline
( ) None	( ) Decantation
( ) Washing	( ) Other
18) Do you ever freeze excess fat for later application?	
( ) Yes	
( ) No	

Figure 1: Survey Questions (continued)

19) What local anesthesia is used at the injection site?	( ) Wetting solution (50 ml of 1% lidocaine plus 1 ml of epinephrine 1:1000 plus 1 liter of normal saline)
( ) 0.5% lidocaine with epinephrine	( ) Epinephrine alone
( ) 1% lidocaine with epinephrine	( ) None
	( ) Other
20) What do you use to inject the fat?	( ) Needle
	( ) Ratchet gun
( ) Cannula	( ) Other
21) What is your estimated volume injected per pass?	( ) 2 - 4 cc
	( ) > 4 cc
( ) < 1 cc	( ) Unknown
( ) 1 - 2 cc	
22) How much do you aim to over-correct?	( ) 10-20%
	( ) 20-30%
( ) None	( ) 30-40%
( ) < 10%	( ) 40-50%
	( ) > 50%
23) In what anatomical locations do you place fat grafts in addition to flap reconstructions? (Mark all that apply)	( ) Intraglandular
	( ) Subglandular
	( ) Pectoral
	( ) Subpectoral
( ) Subcutaneous	( ) Other - Write In (Required)
24) In what anatomical locations do you place fat grafts in addition to implant reconstruction/ -augmentation? (Mark all that apply)	( ) Intraglandular
	( ) Subglandular
	( ) Pectoral
	( ) Subpectoral
( ) Subcutaneous	( ) Other - Write In (Required)
25) In what anatomical locations do you place fat grafts in addition to local defect corrections? (Mark all that apply)	( ) Intraglandular
	( ) Subglandular
	( ) Pectoral
	( ) Subpectoral
( ) Subcutaneous	( ) Other - Write In (Required)
26) What is your estimated total injection volume in addition to flap reconstruction?	( ) 50-100 cc
	( ) 100-150 cc
	( ) 150-200 cc
( ) < 50 cc	( ) > 200 cc
27) What is your estimated total injection volume in correction of local defects?	( ) 100-150 cc
	( ) 150-200 cc
( ) < 50 cc	( ) > 200 cc
( ) 50-100 cc	
28) What is your estimated total injection volume in addition to implant reconstruction/ -augmentation?	( ) 50-100 cc
	( ) 100-150 cc
	( ) 150-200 cc
( ) < 50 cc	( ) > 200 cc
29) Which graft take "enhancing" methods do you use? (Mark all that apply)	( ) BRAVA (preoperatively)
	( ) BRAVA (postoperatively)
	( ) Rigottomies
( ) None	( ) Other - Write In (Required)
30) What is your general opinion on the use of AFG?	( ) I somewhat agree with it
	( ) I am undecided
( ) I strongly agree with it	( ) I somewhat disagree with it
( ) I agree with it	( ) I disagree with it
	( ) I strongly disagree with it
31) What is your perception of volume retention after 6 months?	( ) 50-60%
	( ) 60-70%
( ) < 30%	( ) 70-80%
( ) 40-50%	( ) >80%
32) Do you think the maintenance of volume at 6 months is a result of fat survival, replacement with scar tissue, or a combination?	( ) Fat Survival
	( ) Replacement with scar tissue
	( ) A combination
	( ) Other
33) In your estimation, what is the overall patient satisfaction with the procedure?	( ) Excellent
	( ) Good
	( ) Poor
34) Which one of the following concerns about the use of AFG in breast surgery compares the closest to your own? (Please submit from most to least)	_____Radiological: "The use of AFG in breast surgery impairs future radiological follow-up and breast cancer screening because of the frequent formation of fat necrosis and micro-/ macrocalcifications"
_____Oncological: "The transplantation of adipose-derived-stem-cells and CD34+ progenitors in white adipose tissue poses a risk to promote cancer progression"	_____Practical: "The use of AFG in breast surgery is associated with unacceptable complications such as hematomas, infections and the need for draining oily cysts/ fat necrosis"
35) If you have any additional comments about the use of AFG in breast surgery in general or about this survey, please fill them in below	

Statistical Analyses

The total number of estimated members of the participating countries (NL 425, B 181, D 400, GB 365, F 770, ES 643, IT 473, AT 199, CH 154, GR 271) [21] was 3881. With this, a sample size of 350 is adequate to achieve a confidence level of 95%

with a margin error or confidence interval T5% for the entire population [22]. Continuous data is presented as mean, standard deviation and range. Categorical data is presented as counts or proportions. Differences between baseline characteristics of the respondents from different countries were assessed using t-tests for continuous variables (age) and the KruskalWallis test for ordinal variables (number of years of experience and number of procedures performed per year). Differences between both technical choices and attitude towards fat grafting were assessed in relation to country, years of experience (resident, 0-10, 10-20 and >20 years of experience) and number of procedures performed per year (0-10, 10-20, >30 procedures performed per year). We used logistic regression in case of a binary response variable, ordinal regression in case of an ordinal response variable and multinomial logistic regression in case of multiple response categories.

Table 1: Participating European countries.

Contacted Countries	National Plastic Surgery Association	Active or Passive participation	Number of emails send per country	Response rate
The Netherlands	Netherlands Society for Plastic Surgery, Handsurgery, Aesthetic and Reconstructive Surgery	Active	425	33.2%
Belgium	Royal Belgian Society for Plastic Surgery (RBSPS)	Active	181	23.2%
Austria	Österreichische Gesellschaft für Plastische, Ästhetische und Rekonstruktive Chirurgie (OGPARC)	Active	199	15.0%
Switzerland	Swiss Society of Plastic Reconstructive and Aesthetic Surgery (SGPRAC)	Active	154	10.4%
France	Société Française des Chirurgiens Esthétiques Plasticiens (SOFCEP)	Passive	770	8.4%
United Kingdom	British Association of Plastic, Reconstructive and Aesthetic Surgeons (BAPRAS)	Passive	365	3.8%
Germany	Deutschen Gesellschaft der Plastischen, Rekonstruktiven und Ästhetischen Chirurgen (DGPRÄC)	Passive	400	2.8%
Greece	Hellenic Society for Plastic, Reconstructive and Aesthetic Surgery	Passive	271	2.6%
Spain	Sociedad Española de Cirugia Plastica Reparadora y Estetica (SECPRE) and Asociació de Cirugia Esthetica Plastica (AECEP)	Passive	643	2.2%
Italy	Associazione Italiana di Chirurgia Plastica Estetica (AICPE) and Società Italiana di Chirurgia Plastica Ricostruttiva ed Estetica (SICPRE)	Active	473	1.9%

Results

The participating countries, the method by which survey invitations were send out (passive vs active) and the response-rate is illustrated in table 1. A total of 358 completed questionnaires were retrieved for analysis over a 10 month period (June 2016 – April 2017). Table 2 illustrates the baseline "respondent" demographics. The mean age was 46 years (SD 10.8) with the majority being plastic surgeon (96.9%) next to breast-surgeons (1.7%) and other (1.4%, mostly German gynecologists). Eighty percent were consultants, with a majority having more than 20 years of practicing experience. Ninety percent disclosed having practiced AFT for general purposes (33.5%) or in addition to breast-surgery (56.7%). The majority performed AFT alone (66.2%) in <10 (26.5%) or between 10-30 (38.5%) procedures per year and the vast majority considered him- or herself to be either experienced (41.6%) or moderately experienced (40.5%).

Table 2: Baseline Characteristics

Question/ Variable	Outcome: mean (%)								Missing (%)
Age	46 ±10.8								-
Specialty:									-
- Plastic Surgeon	347 (96.9)								
- Breast Surgeon	6 (1.7)								
- Other	5 (1.4)								
Training:									
- Resident (per yrs of training)		**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**	**Other**	
	57 (15.9)	5 (1.4)	5 (1.4)	7 (2.0)	11 (3.1)	8 (2.2)	15 (4.2)	6 (1.7)	-
- Registered Medical Specialist (experience)		**<5 yrs**	**5-10 yrs**		**10-15 yrs**	**15-20 yrs**	**>20 yrs**		
	288 (80.4)	42 (11.7)	62 (17.3)		47 (13.1)	44 (12.3)	92 (25.7)		1 (0.3)
- Other	12 (3.4)								
AFG Familiarity:									1 (0.3)
- Familiar with AFG in General but not for breast proc.	120 (33.5)								
- Familiar with AFG in General and for breast proc.	203 (56.7)								
- Not familiar with AFG (never practiced)	34 (9.5)								
No. of AFG proc per year:	**<10**		**10-30**		**30-50**		**>50**		35 (9.8)
	95 (26.5)		138 (38.5)		48 (13.4)		42 (11.7)		
Perform AFG alone or with colleague	**Alone**		**With colleague**		**With Senior colleague**		**With Resident**		36 (10.1)
	237 (66.2)		23 (6.4)		30 (8.4)		32 (8.9)		
Experience (self-assessment)	**Experienced**		**Moderately Experienced**		**Moderately Unexperienced**		**Unexperienced**		36 (10.1)
	149 (41.6)		145 (40.5)		19 (5.3)		9 (2.5)		

Technique

The harvest-locations most often used were the abdomen (78.8%), the thigh (56.7%) and the flank (55.6%), with most respondents using wetting solution (50 ml of 1% lidocaine plus 1 ml of epinephrine 1:1000 plus 1 liter of normal saline) as their primary choice for harvest site infiltration (Table 3). Harvesting of fat was mostly performed by way of an liposuction-device (41.9%) preferably through 3mm cannulas (41.1%). When manual-aspiration was used for harvesting (14.0%) most respondents did not know the actual diameter size of the cannula/ -needle. For preparation most respondents performed centrifugation (38.8%) besides washing of the fat (21.2%). Seventy-five percent of respondents used a cannula to re-inject the fat, aiming at 1-2 cc (30.7%) or >4 cc (21.5%) of volume per pass. Overcorrection was used by most respondents (80.5%) ranging from 20-30% (28%) to more than 50% (3.1%). In breast-surgery, more than half (52%) of the respondents grafted the subcutaneous plane in addition to both flap and implant reconstructions as well as the correction of local defects. For flap-reconstructions other planes most commonly grafted were the subglandular (31.8%) and the pectoral (29.9%) spaces with more than half of the respondents aiming at a total grafted volume of 50-100cc (36.2%) or 100-150cc (24.1%). For implant reconstruction/ -augmentation and for local defect correction (LDC) the preferred planes of reinjection were the pectoral (21.8%) vs subglandular (20.9%) and the intraglandular (29.9%) vs subglandular (29.1%) spaces respectively. Methods for AFT take enhancement varied from none (33.8%) to pre- and postoperative use of the Breast Enhancement and Shaping System (BRAVA) in a few select cases (7.5% and 6.1% respectively) and rigottomies (21.5%).

Table 3: AFG Technique

Question/ Variable	Outcome: mean (%)						Missing (%)
Harvest location*	**Gluteal**	**Thigh**	**Flank**	**Abdomen**	**Knee**	**Other**-	
	25 (7.0)	203 (56.7)	199 (55.6)	282 (78.8)	92 (25.7)	15 (4.2)	
Anesthesia at Harvest Location	**0,5% Lido. + Epi**	**1% Lido. + Epi**	**Wetting Solution**	**Epinephrine**	**Other**		37 (10.3)
	24 (6.7)	37 (10.3)	186 (52.0)	26 (7.3)	48 (13.4)		
Harvesting Technique	**Cannula + constant suction**	**Coleman Technique (with Micro-Cannula)**	**Syringe + large-bore Needle**	**Other**			37 (10.3)
	150 (41.9)	98 (27.4)	50 (14.0)	23 (6.4)			
Harvest Cannula diameter:							
- Liposuction Device	**1 mm**	**2 mm**	**3 mm**	**4 mm**	**Unknown**	**Other**	37 (10.3)
	24 (6.7)	72 (20.1)	147 (41.1)	39 (10.9)	25 (7.0)	14 (3.9)	
- Syringe	**14 Gauge**	**16 Gauge**	**18 Gauge**	**Unknown**	**Other**		43 (12.0)
	43 (12.0)	64 (17.9)	40 (11.2)	147 (41.1)	21 (5.9)		
Fat Preparation	**None**	**Washing**	**Centrifugation**	**Adding Insuline**	**Decantation**	**Other**	53 (14.8)
	12 (3.4)	76 (21.2)	139 (38.8)	2 (0,6)	47 (13.1)	29 (8.1)	
Freeze fat (y/n)	**Yes**			**No**			37 (10.3)
	10 (2.8)			311 (86.9)			
Anesthesia at Injection site	**0,5% Lido. + Epi**	**1% Lido. + Epi**	**Wetting Solution**	**Epinephrine**	**None**	**Other**	45 (12.6)
	19 (5.3)	68 (19.0)	34 (9.5)	3 (0.8)	162 (45.3)	27 (7.5)	
Method of Injection	**Cannula**	**Needle**	**Ratchet Gun**	**Other**			38 (10.6)
	268 (74.9)	45 (12.6)	1 (0.3)	6 (1.7)			
Estimated volume of injection per pass	**<1 cc**	**1-2 cc**	**2-4 cc**	**>4 cc**	**Unknown**		38 (10.6)
	68 (19.0)	110 (30.7)	43 (12.0)	77 (21.5)	22 (6.1)		
Overcorrection (aim)	**None**	**<10%**	**10-20%**	**20-30%**	**30-40%**	**40-50%**	**>50%**	40 (11.2)
	30 (8.4)	32 (8.9)	96 (26.8)	99 (27.7)	40 (11.2)	10 (2.8)	11 (3.1)	
Grafted anatomical planes per indication*:	**Subcutaneous**	**Intra-glandular**	**Sub-glandular**	**Pectoral**	**Sub-pectoral**	**Other**	
- Flap reconstructions	186 (52.0)	83 (23.2)	114 (31.8)	107 (29.9)	43 (12.0)	12 (3.4)	
- Implant reconstruction/ -augmentation	186 (52.0)	66 (18.4)	75 (20.9)	78 (21.8)	25 (7.0)	7 (2.0)	
- Local defect corrections	186 (52.0)	107 (29.9)	104 (29.1)	78 (21.8)	32 (8.9)	8 (2.2)	
Estimated total injection volume per indication:	**<50 cc**	**50-100 cc**	**100-150 cc**	**150-200 cc**	**>200 cc**		
- Flap reconstructions	21 (10.6)	72 (36.2)	48 (24.1)	42 (21.1)	16 (8.0)	159 (44.4)	

Question/ Variable	Outcome: mean (%)					Missing (%)
- Implant reconstruction/ -augmentation	39 (19.6)	73 (36.7)	44 (22.1)	31 (15.6)	12 (6.0)	
- Local defect corrections	39 (19.6)	95 (47.7)	47 (23.6)	17 (8.5)	1 (0.5)	
AFG enhancement*	**None**	**BRAVA preop**	**BRAVA postop**	**Rigottomies**	**Other**	
	121 (33.8)	27 (7.5)	22 (6.1)	77 (21.5)	8 (2.2)	

Abbreviations: Lido. (Lidocaine), Epi (Epinephrine), Wetting Solution (50 ml of 1% Lidocaine + 1 ml of epinephrine 1:1000 plus 1 liter of Saline), BRAVA (Breast Enhancement and Shaping System®), preop (preoperatively), postop (postoperatively),
*: Multiple answers possible

Attitude

The vast majority of respondents strongly agreed (47.8%) or agreed (38.0%) with the use of AFT for appropriate indications (see Table 4) with an almost equal distribution of respondents estimating the volume retention after 6 months to be in the range of 40-50% (23.5%), 50-60% (21.8%) or 60-70% (28.2%). There was a clear division in the opinion about causative factors when it comes to volume retention with approximately half of respondents attributing the results to fat-survival (50%) or a combination of fat survival and scar-tissue replacement (41.9%). Patient-satisfaction as estimated by the surgeon was either excellent (51.4%) or good (39.7%) in the majority of respondents.

Table 4: AFG opinion

Question/ Variable	Outcome: mean (%)							Missing (%)
General opinion (agreement with AFG)	**Strongly Agree**	**Agree**	**Somewhat Agree**	**Undecided**	**Somewhat Disagree**	**Disagree**	**Strongly Disagree**	6 (1.7)
	171 (47.8)	136 (38.0)	28 (7.8)	6 (1.7)	8 (2.2)	1 (0.3)	2 (0.6)	
Estimated volume retention >6 months	**<30%**	**40-50%**	**50-60%**	**60-70%**	**70-80%**	**>80%**		5 (1.4)
	47 (13.1)	84 (23.5)	78 (21.8)	101 (28.2)	33 (9.2)	10 (2.8)		
Estimated cause of volume retention	**Fat Survival**	**Replacement with scar tissue**	**Combination (fat survival + scar tissue replacement)**	**Other**				6 (1.7)
	179 (50.0)	9 (2.5)	150 (41.9)	14 (3.9)				
Estimated patient satisfaction with AFG	**Excellent**	**Good**	**Poor**					5 (1.4)
	184 (51.4)	142 (39.7)	27 (7.5)					

Differences between countries, surgeon-experience and aft procedure performed per year

Due to the small numbers of respondents for most participating countries (D, GB, ES, IT, CH, GR) a comparison could only be made between the Netherlands, Belgium, France and Austria with the remaining countries pooled together as "other". Furthermore, since no consensus and therefore golden-standard currently exists regarding AFT-technique, no deviation thereof with regard to the various countries analyzed, can be calculated. Therefore, the largest group of respondents (NL) was considered as an arbitrary baseline (see Table 5a).

Table 5a: Differences between countries

	Netherlands (baseline) [1]	Belgium	France	Austria	Other
No. of Respondents (%)	**141 (39.4)**	42 (11.7)	65 (18.2)	30 (8.4)	80 (22.3)
Mean age ± SD	**42 ± 10**	46 ± 11 ↑*	51 ± 10 ↑***	45 ± 10 ↑ns	50 ± 10 ↑***
Experience (%):					
- Resident	**32.8**	5.0	0.0	10.3	9.5
- Specialist (0-10 yrs)	**43.3**	40	23.4	41.4	18.9
- Specialist (10-20 yrs)	**21.9**	27.5	28.1	31.0	31.1
- Specialist (>20 yrs)	**10.9**	27.5 ↑***	48.4 ↑***	17.2 ↑*	40.5 ↑***
AFT proc./ year (%):					
- <10	**47.9**	15.0	18.5	20.0	21.1
- 10-30	**38.5**	47.5	35.4	48.0	51.3
- >30	**13.7**	37.5 ↑***	46.2 ↑***	32.0 ↑*	27.6 ↑***
Harvest location (%)					
- Thigh	**55.3**	50.0 ↓*	72.3	56.7	50.0 ↓*
- Abdomen	**75.2**	78.6	81.5↓ *[2]	70.0	86.3
Local (donor site) anesthesia (%)					
- Wetting solution	**69.8**	50.0	34.4 ↓***	64.0	61.8
Harvesting Technique (%)					
- Liposuction device	**65.5**	57.5	39.1 ↓***	28.0 ↓***	52.6 ↓*
Liposuction Cannula (%)					
- < 2mm	**43.8**	30.8	24.2	13.0	39.1
- > 3mm	**56.2**	69.2	75.8 ↑**	87.0 ↑**	60.9
Preparation (%)					
- Washing	**27.6**	31.3	22.8	20.0 ↓*	21.3
- Centrifugation	**44.0**	43.8	68.4 ↑**	16.0 ↓**	41.3
Estimated volume per pass (%)					
- <1 cc	**26.5**	20.5	5.1	12.5	36.5
- 1-2 cc	**46.1**	38.5	15.3	54.2	35.1
- >2 cc	**27.5**	41.0	79.7 ↑***	33.3	28.4

	Netherlands (baseline) [1]	Belgium	France	Austria	Other
Overcorrection (%)					
- None	**10.3**	0.05	4.7	20.0	11.0
- <20	**42.2**	42.5	32.8	32.0	45.2
- 20-30	**26.7**	35.0	37.5	32.0	30.1
- >30	**20.7**	17.5	25.0 ↑*	16.0	13.7
AFT + Flap reconstruction; injection planes (%)					
- Subcutaneous	**54.6**	52.4	46.2 ↓**	53.3	51.2 ↓*
- Intra-glandular	**25.5**	35.7	26.2	0.0	18.8 ↓*
- Sub-pectoral	**7.1**	19.0	27.7 ↑*	3.3	7.5
AFT + Implant reconstruction/ augmentation; injection planes (%)					
- Subcutaneous	**55.3**	52.4 ↓*	47.7 ↓***	50.0	50.0 ↓**
AFT + Local defect corrections; injection planes (%)					
- Subcutaneous	**53.9**	52.4	46.2 ↓**	56.7	51.3 ↓*
- Intra-glandular	**38.3**	38.1	24.6 ↓**	16.7 ↓*	20.0 ↓***
AFT + Flap reconstruction; estimated total injection volume					
- <100	**62.4**	30.4	9.4	47.1	52.4
- 100-150	**25.9**	26.1	15.6	35.3	21.4
- >150	**11.8**	43.5 ↑*	75.0 ↑***	17.6	26.2

The arrow (↓, ↑) indicates the value in which the country differs from the baseline (↓=lower/ less, ↑=higher/ more).

Significance:

ns P > 0.05 ** P ≤ 0.01

* P ≤ 0.05 *** P ≤ 0.001

[1]: Arrows in the columns depict significant deviations from the column "Netherlands", which serves as the baseline.

[2]: Percentages are based on the data, significance levels are based on model estimates. Discrepancies between differences between percentages and the direction of the arrows are due to correction for other variables in the model.

The mean age of the Dutch respondents was significantly lower than that of other countries. The years of experience and number of AFT-procedures performed yearly were higher in Belgium, France, Austria and the other countries combined. Considering, harvest-locations, the thigh was significantly less used in Belgium and in the other countries combined and French respondents were less inclined to use the abdomen compared to the Dutch. The French and Austrian respondents seemed to prefer manual-aspiration over a liposuction-device and larger over smaller cannula-sizes (>3 vs <2 mm) compared to the Dutch respondents. Furthermore, centrifugation was performed significantly more by the French and both centrifugation as well as washing significantly less by the Austrian surgeons, respectively. In addition to both flap- and implant (breast) reconstruction as well as in correcting local (mammary) defects the French re-

spondents performed significantly less AFT in the subcutaneous plane, compared to the Dutch. In addition, so did both the French and the Austrian respondents when it came to intra-glandular AFT for LDC. On the contrary, in addition to flap (breast) reconstructions, the French, performed significantly more sub-pectoral fat injections. Finally, when asked about the amount of injected fat both the French and the Belgian surgeons injected significantly more in addition to flap-reconstruction than the Dutch surgeons.

Tables 5b and 5c stratify the number of respondents based on their experience and number of AFT-procedures performed yearly. What stands out is both the harvesting-location as well as technique and –cannula-size, besides the estimated injected volume. For example, we see that the flank as a harvesting-location is more utilized by surgeons who perform more AFT-procedures yearly, but is used less by surgeons with more overall clinical experience. On the contrary, the use of a liposuction-device is less often used by both less experienced surgeons as well as surgeons who perform more AFT-procedures per year. When looking at the different injection planes used, compared to the number of AFT-procedures performed yearly, there seems to be a direct relationship between the two for all injection planes. In other words, the higher the numbers of AFT-procedures performed yearly, the more injection planes are utilized by the surgeon. This holds true for intra-glandular injections as well.

Table 5b: Outcome per years of overall experience

	(Residents)[1]	<10	10-20	>20
No. of Respondents (%)	**57 (15.9)**	104 (29.1)	91 (25.4)	92 (25.7)
Harvest location (%)				
- Flank	**47.3**	59.6 ↓*[2]	65.9 ↓*[2]	48.9 ↓**[2]
Harvesting Technique (%)				
- Liposuction device	**47.1**	47.5 ↑*	52.2 ↑*	62.9 ↑**
Liposuction Cannula (%)				
- <2 mm	**18.2**	30.3	31.7	44.4 ↑*
- >3 mm	**81.8**	69.7	68.3	55.6
Estimated volume per pass (%)				
- <1 cc	**23.1**	13	25.0	31.3
- 1-2 cc	**38.5**	50.0	33.0	26.5
- >2 cc	**38.5**	37.0	42.0	42.2

The arrow (↓, ↑) indicates the value in which the country differs from the baseline (↓=lower/ less, ↑=higher/ more).
Significance:
ns P > 0.05
* P ≤ 0.05

** P ≤ 0.01
*** P ≤ 0.001
1: Arrows in the columns depict significant deviations from the column "Residents"
2: Percentages are based on the data, significance levels are based on model estimates. Discrepancies between differences between percentages and the direction of the arrows are due to correction for other variables in the model.

Table 5c: Outcome per AFT procedures performed yearly

	<10 proc./ year [1,2]	10-30 proc./ year	>30 proc./ year
No. of Respondents (%)	**95 (26.5)**	138 (38.5)	90 (25.1)
Harvest location (%)			
- Flank	**50.5**	61.6 ↑*	73.3 ↑**
Harvesting Technique (%)			
- Liposuction device	**67.4**	50.0 ↓*	43.2 ↓*
AFT + Flap reconstruction; injection planes (%)			
- Subcutaneous	**53.7**	57.2	62.2 ↑*
- Sub-glandular	**23.2**	34.8	48.9 ↑**
- Pectoral	**20.0**	30.4	51.1 ↑***
AFT + Implant reconstruction/ augmentation; injection planes (%)			
- Intra-glandular	**15.8**	18.1	28.9 ↑*
- Sub-glandular	**15.8**	23.2	31.1 ↑*
- Pectoral	**16.8**	21.7	35.6 ↑*
AFT + Local defect corrections; injection planes (%)			
- Subcutaneous	**52.6**	55.8	65.6 ↑**
- Sub-glandular	**26.3**	29.7	42.2 ↑*
- Pectoral	**11.6**	22.5	40.0 ↑***

The arrow (↓, ↑) indicates the value in which the country differs from the baseline (↓=lower/ less, ↑=higher/ more).
Significance:
ns P > 0.05
* P ≤ 0.05
** P ≤ 0.01
*** P ≤ 0.001
[1]: Arrows in the columns depict significant deviations from the column "<10 proc./ year"
[2]: Percentages are based on the data, significance levels are based on model estimates. Discrepancies between differences between percentages and the direction of the arrows are due to correction for other variables in the model.

Discussion

With the growing popularity of AFT amongst plastic surgeons the number of AFT-techniques and subsequently the patented AFT devices currently commer-

cially available, increases. The obvious attraction of the technique for both patients and surgeons comes forth from the desire to recycle fat tissue for a beneficial – often defect occupying – goal in reconstructive or augmentational surgery. Hence, the high surgeon- and patient- satisfaction rates that are generally reported in clinical studies and systematic reviews [23,24]. However, critics of AFT have strong arguments in pointing out the disadvantages such as uncertainty regarding oncological- and radiological-safety in breast-reconstruction/ augmentation, besides unpredictable long-term results. In the UK, Germany and France, clinical-guidelines are now available to standardize the technique, aiding both clinical-practice and reproducibility amongst scientific-studies. In this light an overview of real-time clinical-practice of AFT in Europe identifying differences between countries might aid further scientific studies in the search for the golden-standard in AFT.

Despite an adequate overall response-rate we found a low response-rate per country which may have been attributable to the headline of the survey invitation. This revealed the technical aspect of some of the questions, which might have discouraged surgeons who never practice AFT to respond. More than a quarter of the respondents had >20 years of practicing experience and higher rates of these more experienced surgeons were found in all of the other countries compared with the Netherlands. This was probably attributable to the higher number of residents amongst the Dutch respondents. Our survey showed that breast-surgery is still the most prominent indication for AFT in Europe. Also, the majority of surgeons performed AFT alone, conform the findings of Skillman et al [17], showing that while AFT can be time-consuming, it is not a two-man's job necessarily. While AFT is a popular procedure, it is still not practiced often, with 26.5% of respondents performing less than 10 AFT-procedures per year and only 11.7% performing more than 50. These findings are in line with Kaufman et al [16] and although a longer learning curve might be the result of the relative few procedures performed, most surgeons considered themselves experienced.

The technique used remains one of the most heterogenic aspects of AFT and while factors like harvesting-technique and preparation seem to be rather uniform with the Coleman-technique [25,26], deviations thereof are becoming apparent. The abdomen is still the most prominent harvesting-location overall. Second to this is the flank with even higher rates in the subgroup of respondents who perform more AFT-procedures. In 2017 Europe, the vast majority (41.9%) of surgeons is using a liposuction-device which might be attributable to the time-saving properties of this technique. The French and the Austrian respondents

used a liposuction-device significantly less often than the Dutch population, which we hypothesized as possibly due to the higher level of experience (and Coleman Technique adherence) of respondents from these countries. While randomized-controlled-trials comparing both methods are clearly needed, the recent systematic review by Shim et al. [27], indicated a slight preference for manual-aspiration, based on several small-cohort, retro- and prospective-studies [28-31]. The preferred cannula-size when using a liposuction-device was 3mm in 41%, with an equal percentage of respondents indicating not knowing the cannula-size when using manual-aspiration. This seems to be an area where improvement can be achieved, since several studies have indicated that the size of both the aspiration and injection-cannula (>3mm - < 6mm) matter significantly in terms of adipocyte-viability [32,33]. Finally, in terms of injection-technique and –planes, half of the respondents aimed at injecting <1 to 2cc of fat, while over-correcting 10-30% in-line with the Coleman-Method, with only the French injecting more. With regard to breast-surgery, when AFT is used in addition to flap-reconstruction, implant-reconstruction or augmentation and LDC, the subcutaneous plane was grafted most, followed by the subglandular and pectoral planes. What is interesting to see is that the intra-glandular plane was grafted for all indications ranging from 18.4% in addition to implant-reconstruction, to 30% in LDC. Even more interesting is the fact that intraglandular injection rates also seemed to be higher in more experienced surgeons based on the number of AFT-procedures performed. Both the British and German clinical-guidelines [34,35], currently strongly advise against the utilization of intra-glandular AFT because of the possible carcinogenic differentiation of (remaining dormant) breast (cancer) cells [36-38]. While the number of respondents from the UK and Germany were too low to make any comparisons between countries, the Dutch plastic surgery association (NVPC) advises its members to adhere to the British guidelines and otherwise to keep up-to-date on the most recent scientific literature when performing AFT. The authors presume the same holds true for other countries but nonetheless, there seems to be a gap between what is recommended and what is actually performed and herein might lay certain benefits from proper surgeon-education when it comes to oncological safety of AFT.

The overall approval of the respondents with AFT in general as well as the surgeon perceived patient-satisfaction was considered high and seems in line with recent studies. The perception of what causes the eventual volume-retention was either fat-survival or a combination thereof with scar tissue formation, and further histological animal-studies, preferably with long-term follow-up are

needed to substantiate the answer to this question. Finally, concerns with AFT in breast-surgery mainly concern; oncological-safety, radiological-safety or practical-issues. Figure 2 highlights the order in which these concerns troubled the respondents, illustrating that further studies should focus on the oncological and radiological-safety of the technique.

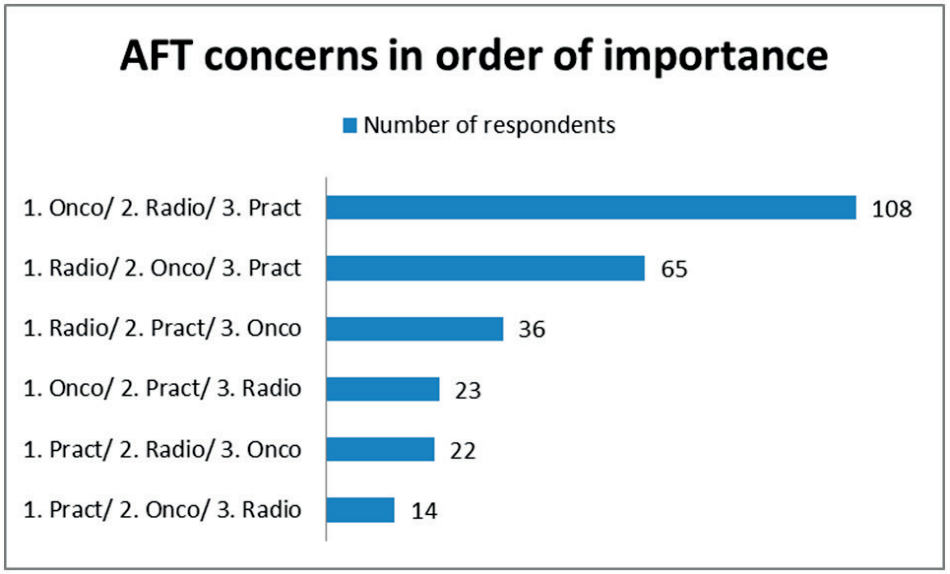

Figure 2: Respondents concerns with the practice of AFT in order of most clinically important

Onco: "The transplantation of adipose derived-stem-cells and CD34+ progenitors in white adipose tissue poses a risk to promote cancer progression"
Radio:"The use of AFG in breast surgery impairs future radiological follow-up and breast cancer screening because of the frequent formation of fat necrosis and micro-/ macro-calcifications"
Pract:"The use of AFG in breast surgery is associated with unacceptable complications such as hematomas, infections and the need for draining oily cysts/ fat necrosis"

Limitations

The information gathered by survey-studies is dependent on honest answers. While the authors trust the intentions of the respondents, the accuracy of the answers given can – on a subconscious level – be colored by embarrassment, lack-of-memory, alacrity or even boredom [39]. Furthermore, discrepancy between responders and non-responders can create a selection bias. Finally, while the questions leave little room for interpretation, certain options like "somewhat-agree" can mean different things to different individuals. Nonetheless, for the

first time we were able to highlight differences in AFT-technique between countries and level of experience and point out the ongoing practice of intraglandular fat grafting in conjunction with breast-surgery.

Conclusion

This study provides the first overview of clinical practice regarding AFT in Europe and highlights important differences between countries that can aid in the focus of future studies as well as point out discrepancies in the physician adherence to clinical guidelines. The overall experience with AFT amongst respondents was moderate to high, with most applying its use in addition to breast-surgery. Coleman's method is still the most widely used AFT-technique but deviations thereof lay in the areas of harvesting technique and cannula-sizes. The injection-planes of AFT, in addition to breast-surgery, are in order of most-used; the subcutaneous, subglandular and pectoral planes. However, despite prominent discouragement of the British and German clinical-guidelines, intraglandular AFT still occurs in clinical practice today and this should be the focus of further surgeon-education in Europe.

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Conflict of interest statement

None.

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Chapter 8

Autologous Fat Transfer after Augmentation and Reconstruction of the Female Breast; An International, Cross-sectional Photo-Comparison study amongst different Physician-, and Patient Study Groups

Groen JW, Piatkowski AA, Sawor JH, Wilschut JA, Khouri RK, van der Hulst RRJW, Ritt MJPF. Submitted

Abstract

Background: Autologous-Fat-Transfer (AFT) is a rapidly evolving technique in plastic surgery, with innovative articles published monthly and new techniques developed at a great pace. Despite this upsurge in scientific/clinical interest, objectifying satisfaction has only recently progressed beyond simple likert-/visual analog-scales. Furthermore, differences in satisfaction between patients and surgeons has not been thoroughly studied.

Method: A photo-comparison-study between European plastic surgeons and different patient-groups (1. DIEP-reconstruction, 2. Cosmetic-Augmentation, 3. Control-group) was conducted to investigate agreement between groups. Three sets of pre-/postoperative photographs illustrating patients treated with Breast-Enhancement-and-Shaping-System (BRAVA) + AFT for various indications in breast-surgery, were scored according to the Harris-Scale and interrater-agreement was analyzed using Cohen's Kappa.

Results: The overall agreement between the surgeons and the groups of augmentation-, control group- and DIEP patients was fair, moderate and substantial, respectively. Interrater-agreements amongst different patient-groups/ and surgeons from different countries amongst themselves was substantial to almost perfect. Finally, we found that patients are generally more optimistic about postoperative results than surgeons.

Discussion: In our study, augmentation patients showed the lowest agreement with surgeons, in the cosmetic appreciation of BRAVA+AFT and this group might benefit from a more thorough preoperative consultation regarding expectations when choosing AFT. However, overall patients tend to be more optimistic about postoperative results and patient-education in general does not seem influenced by surgeon nationality.

Conclusion: There are significant differences between surgeons and patients in the cosmetic evaluation of BRAVA+AFT and further studies should focus on the qualitative aspects of these differences to further balance patient and surgeons expectations.

Introduction

Autologous fat transfer (AFT) is becoming increasingly popular in various aspects of plastic surgery. Concerning the female breast, the first description dates from 1893 with Neuber attempting transfer of bulk volumes of fat [1]. Since then, other notable developments have been the advent of liposuction with Bircoll, in 1987, describing the injection of autologous fat, to the breast [2-4] and the prohibition of its use, the same year, by the American Society of Plastic Surgeons (ASPS) because of the possible carcinogenic effects and the induction of radiographic changes that could impede future diagnostics [5]. Furthermore, with the first standardized protocol described by Coleman in 1995 [6] leading to an increase in the number of objective and reproducible study-designs the Fat Graft Task Force of the American society of Plastic surgeons, in 2009, stated that the procedure was no longer prohibited [7]. This resulted in large volume studies, systematic reviews and meta-analysis, showing the efficacy and safety in terms of improving volume retention and acceptable oncological and radiological safety respectively [8-12]. With this gradual reassurance of the safety of the technique, the authors believe the aim for further research is to lean more towards efficacy since this is an area where profit is still to be gained. Some studies describe volume retention but in a heterogenetic way. Moreover, patient satisfaction is being described occasionally, and only recently with the use of validated questionnaires like the Breast-Q [13-16]. Also, the satisfaction of patients and surgeons is generally reported in rates, and comparisons in the cosmetic appreciation of the procedure between groups of surgeons and patients based on background and experience has not been thoroughly studied. Finally, in a recent conducted European survey study, performed by the same authors, the surgeon satisfaction in general (based on their own experience with AFT) did not differ between countries, however it is interesting to see if the same holds true in the cosmetic evaluation of AFT on a pre-/ postoperative photographical basis for different breast surgery indications.

Therefore, the aim of this study is to report on the interrater agreement between European surgeons mutually as well as between surgeons and different groups of patients in the cosmetic evaluation of patients treated with the Breast Enhancement and Shaping System® (BRAVA) +AFT.

Methods

An extensive international, cross-sectional, observational, photo-comparison study amongst European plastic-/ breast surgeons and Dutch patients was conducted. The photographs were collected from a high-volume center in the US (Miami Breast Center, US, courtesy of Dr. R. Khouri) and displayed the pre- and postoperative appearance of breasts treated for reconstruction or augmentation purposes, shot in direct antero-posterior (AP) and bilateral oblique (BO) direction. The photographs were stripped of any information that might identify the patient and were presented using an online questionnaire (Survey Gizmo® (Boulder, CO) supplemented with a brief explanatory text of the procedures leading up to the postoperative effect (see Figures 1-3).

The respondents were asked to score pre-/ and postoperative photographs of patients treated with AFT + BRAVA for various indications (e.g. breast augmentation, breast reconstruction and contour defects) using the Harris Scale (HS); excellent, good, fair or poor.

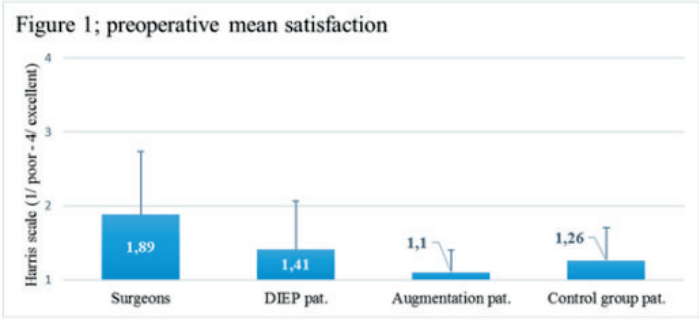
Plastic surgeons from ten European countries (Netherlands, Belgium, Germany, Great Britain, France, Spain, Austria, Switzerland, Italy and Greece) were contacted either directly through their national professional organization or indirectly by email with an invitation to score the pre- and postoperative photographs. A reminder was sent by email after two weeks. In addition to the physician rating, patients from two local hospitals (VieCuri Medical Center, Venlo/ Zuyderland Medical Center, Sittard, Limburg, The Netherlands) were contacted according to the ethical guidelines from the Maastricht University Medical Center and asked for participation in this study. Three patient groups were studied and contacted as follows.

- Group 1: Comprised of female patients treated by Deep Inferior Epigastric Artery Perforator reconstruction in the period 2014-2016, with or without additional AFT
- Group 2: Comprised of female patients who underwent breast augmentation, in the period of 2014 up to April 2017.
- Group 3: Functioned as the control group and was comprised of female patients not previously treated (either surgically or otherwise) for breast related pathology.

Figure 1: Pre- and postoperative appearance of BRAVA + AFG in total bilateral breast reconstruction and mean satisfaction scores (Harris Scale)

Preoperative photograph:
45 year old woman, 2 months after bilateral mastectomy with radiotherapy on left breast.

Postoperative photograph:
4 years later after 2 bilateral sessions of pre-/ and postoperative BRAVA expansion + AFG sessions and 2 additional sessions of pre-/ and postoperative BRAVA expansion + AFG sessions on left breast only.



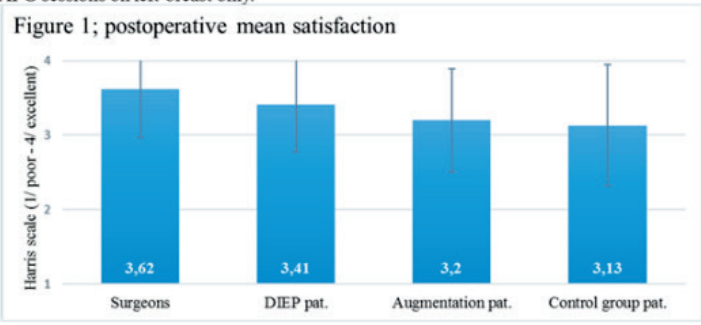
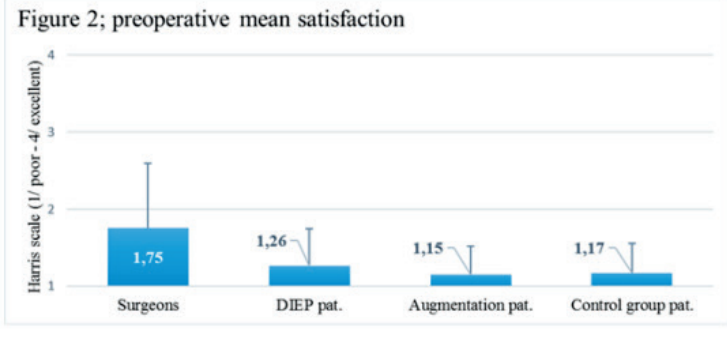


Figure 1: Pre- postop Reconstruction

Figure 2: Pre- and postoperative appearance of BRAVA + AFG in total bilateral breast augmentation

Preoperative photograph:
34 year old woman with bilateral involutional atrophy (congenital small breasts).

Postoperative photograph:
1 year later, after bilateral session of preoperative BRAVA expansion + AFG session (300 ml/ breast).



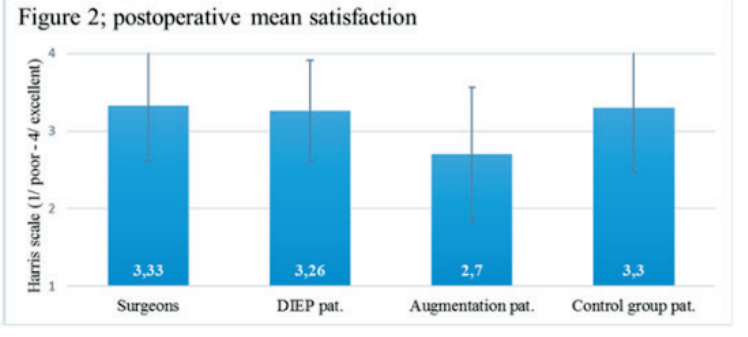


Figure 2: Pre- postop Augmentation

Figure 3: Pre- and postoperative appearance of BRAVA + AFG for local defect correction after lumpectomy

Preoperative photograph:
Non-radiated, left-sided partial mastectomy, with subsequent retracted scar on the lateral side of the left breast.

Postoperative photograph:
6 months after one session of preoperative BRAVA expansion + AFG session (325 ml per breast).

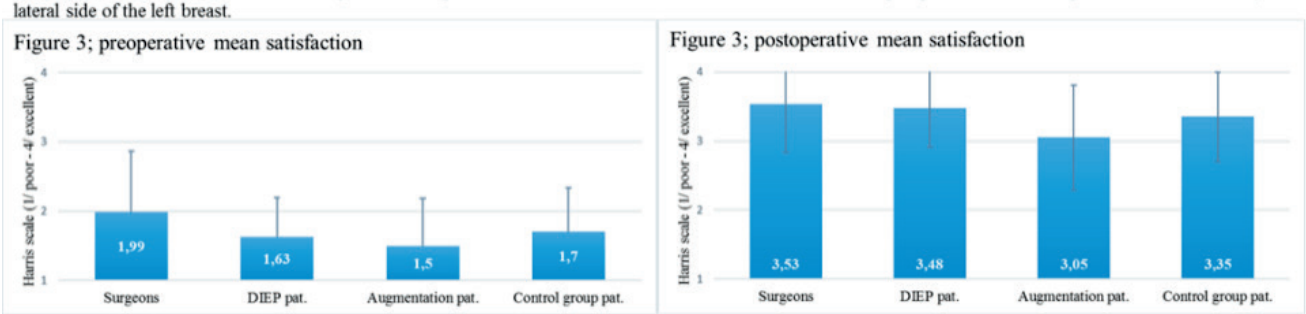


Figure 3: Pre- postop local defect correction

Eligibility criteria

Female patients, between the age of 18 and 70, without previous or current medical training were included when able to understand the implications of the photographs or explanation of the AFT technique (as judged by the investigator). Emotionally unstable patients - due to current or previous breast-cancer related mental trauma (as judged by the investigator) - in who the photographs might aggravate anxiousness or negative emotions were excluded.

Patient recruitment

Group 1 and 2 patients were recruited in a retrospective matter. A recruitment letter was send by the treating physician, in which they were informed of the study and subsequently asked if they may be contacted by phone for further information and possibly inclusion (checkbox yes or no option). Once patient approval was received the patient was contacted by phone by the researcher (JG) and an understandable explanation about the content and methodology of the study was provided, at the end of which the patient was asked for participation in this study. On accordance the patient received the questionnaire including the photographs through an online (Survey Gizmo®) link followed by an informed consent letter with retour envelop send through conventional mail. The photographs were supplemented with an explanatory text of the procedures leading up to the postoperative effect in layman Dutch and the patients were asked to grade the difference according to the HS. Group 3 patients were included in a prospective consecutive manner in which the initial (none breast related) consultation was concluded with the treating physician inquiring if the patient was willing to participate in a study. On agreement the patient was approached by the researcher (JG) in the same clinical setting and recruited in the same matter as the previous patient groups with the only exception that patient information- and informed consent letters were handed-out and subsequently collected physically during the follow-up consultation (minimum of 2 weeks).

Statistical analysis

Agreement between the evaluations of different groups of raters (surgeons, DIEP patients, augmented patients and control patients) was calculated by the agreement index (kappa) suggested by Van belle and Albert (2009) [17,18]. A kappa score equal or below 0 will be considered to indicate poor agreement; 0.01–0.20 slight

agreement; 0.21–0.40 fair agreement; 0.41–0.60 moderate agreement; 0.61–0.80 substantial agreement; 0.81–0.99 almost perfect; and 1.00 perfect agreement. The sampling variance of kappa was determined with the Jackknife method, as suggested by Van belle and Albert (2009) [17,18]. The confidence interval for kappa was derived from the sampling variance. Next to the agreement between different groups of raters, the agreement between surgeons from different countries was assessed in a similar way. In addition to analyzing the interrater agreement between groups on the pre- and postoperative photographs themselves, the authors wanted to examine the agreement on the increase (or possibly decrease) in cosmetic evaluation between the pre- and postoperative photographs, i.e. the scoring trend. Therefore, the difference in the cosmetic evaluation between the pre- and postoperative photographs based on the Harris Score (HS) was calculated for every individual respondent per group. These differences were categorized as follows; (1) negative difference (i.e. postoperative photograph scored lower than preoperative photograph), (2) postoperative HS = preoperative HS +0, (3) postoperative HS = preoperative HS +1, (4) postoperative HS = preoperative HS +2, (5) postoperative HS = preoperative HS +3. Kappa was calculated for the agreement on the scoring trend between the surgeons and the patient groups and the patient groups among each other, for all sets of photographs. The difference in scoring trend per set of pre-/ postoperative photographs between the groups of raters was evaluated by ordinal regression analysis.

Results

A total of 312 plastic surgeons completed the questionnaires out of 520 and these were included for analysis. Despite the fact that surveys were distributed amongst (members of) European plastic surgery associations only, some of the respondents worked outside of Europe. Table 1 illustrates the distribution amongst countries, with most respondents practicing from the Netherlands (37.2%), France (18.9%) or Belgium (11.5%). The mean age of respondents was 45.9 years (SD 10.6) with the majority being plastic surgeon (97.8%) next to breast surgeons (1.6%) and other (0.6%, mostly German gynecologists). Eighty-two percent completed their medical specialty, with a quarter of the respondents having more than 20 years of practicing experience. When asked about familiarity with AFT, 91.3% disclosed having practiced AFT, either for general purposes (32.7%) or in addition to breast surgery (58.7%). Of the active practicing respondents the majority performed AFT alone (73.7%), in <10 (28.4%) or

between 10-30 (43.5%) procedures per year and the vast majority considered him- or herself to be either experienced (48.1%) or moderately experienced (42.8%).

Table 1: Participating countries and patients

Surgeons			
Country	*Active vs Passive participation*	*(Estimated*) emails send per country (response rate)*	*Number of respondents (overall %)*
Netherlands	Active	*425 (33.2%)	116 (37.2)
France	Passive	770 (8.4%)	59 (18.9)
Belgium	Active	*181 (23.3%)	36 (11.5)
Austria	Active	*199 (15.0%)	25 (8.0)
Spain	Passive	643 (2.2%)	15 (4.8)
Switzerland	Passive	154 (10.4%)	14 (4.5)
United Kingdom	Passive	365 (3.8%)	13 (4.2)
Germany	Passive	400 (2.8%)	9 (2.9)
Greece	Passive	271 (2.6%)	7 (2.2)
Italy	Active	*473 (1.9%)	7 (2.2)
Other[1] (Australia, Colombia, Costa Rica, French Polynesia, Ireland, Lebanon)	/		6 (1.9)
United States[1]	/		5 (1.6)
Patients			
Group	*Patients contacted*	*Response rate*	*Age (SD)*
Total	245	101 (41.2%)	50.8 (± 12.3)1
DIEAP reconstruction	112	43 (38.4%)	55.4 (± 9.3)
Breast Augmentation	86	20 (23.3%)	39.1 (± 11.8)
Control group	47	38 (80.9%)	51.9 (± 11.9)

[1:] Respondents from outside Europe were unintendedly collected through membership of a European association

Approximately one-hundred patients, out of 245 (41.2%), responded and were included in the final analysis. There were a total of 43 DIEP patients, next to 20 patients after breast augmentation and 38 control patients. The response rate between groups ranged from 23.3 percent in the augmentation group to 80.9 percent in the control group. The mean age overall was 50.8 (SD 12.3) years, with DIEP- (55.4/ SD 9.3) and control (51.9/ SD 11.9) patients both being significantly older than the augmentation group (39.1/ SD 11.8), p-value <0.001.

Interrater agreement: surgeons - patients

The interrater agreement between the total group of surgeons and the total group of patients over all sets of photographs was considered moderate (0.45-0.55). The interrater agreement between the surgeons and the DIEP patients over all sets of photographs was substantial with a kappa of 0.63 (95% CI:0.49-0.76) but a moderate agreement was found in the evaluation of the results of BRAVA + AFT for breast reconstruction (fig 1/ table 2). The interrater agreement between the surgeons and the group of augmentation- and control group patients over all sets of photographs, was considered fair and moderate, with kappa's of 0.36 (95% CI:0.23-0.48) and 0.51 (95% CI:0.38-0.63), respectively. In addition a moderate agreement (kappa 0.45) was found between the surgeons and the augmentation group on the evaluation of BRAVA + AFT for local defect corrections (fig 3/ table 2) besides a fair interrater agreement (kappa 0.39) between the surgeons and the control group on evaluating BRAVA + AFT in breast reconstruction (fig/ table 2).

Interrater agreement: between patient groups

In comparing the different patient groups we found a substantial interrater agreement over all sets of photographs between the group of DIEP patients and augmentation patients (kappa 0.69/ 95% CI:0.56-0.81) and the group of augmentation patients and control group patients (kappa 0.75/ 95% CI:0.61-0.89), respectively. Furthermore, an almost perfect interrater agreement was found between the group of DIEP patients and the control group, with a kappa of 0.82 (95% CI:0.74-0.90).

Table 2: Interrater agreements scores per set of (pre-/ postoperative) photographs, amongst groups

	Total	Set 1	Set 2	Set 3
Surgeons vs patients total	0,555	0,458	0,555	0,685
Surgeons vs DIEP patients	0,629	0,540	0,629	0,728
Surgeons vs Augmentation patients	0,357	0,309	0,353	0,436
Surgeons vs Control group patients	0,509	0,393	0,512	0,690
DIEP patients vs Augmentation patients	0,685	0,690	0,692	0,634
DIEP patients vs Control group patients	0,822	0,769	0,808	0,885
Augmentation patients vs Control group patients	0,748	0,738	0,781	0,690

Fair agreement; **Moderate agreement**; **Substantial agreement**; **Almost perfect agreement**.

Scoring trend: difference between pre- and postoperative photograph

Only very low or negative interrater agreements between all groups (patients-patients and patients-surgeons) were found when looking at scoring trends i.e.; increase (or possibly decrease) in cosmetic evaluation between the pre- and postoperative photographs per set. Ordinal regression analysis shows that the patient groups are generally more optimistic about the improvement than the surgeons, with significant differences between the DIEP patients and the surgeons ($p=0.042$) for the first set of photographs (fig 1), and between both DIEP and control patients compared to the surgeons ($p=0.003$ and $p=0.004$) for the second set of photographs (fig 2).

Interrater agreement: surgeons per country

The interrater agreement between surgeons from four different European countries over all sets of photographs ranged from substantial to almost perfect. A substantial interrater agreement was found in comparing the cosmetic evaluation of surgeons from the Netherlands with the evaluation of surgeons from France, Austria and Belgium with kappa's of 0.73 (95% CI:0.59-0.87), 0.79 (95% CI:0.68-0.91) and 0.73 (95% CI:0.62-0.85), respectively. Furthermore, a substantial interrater agreement (kappa 0.70/ 95% CI:0.57-0.82) was found between surgeons from Belgium and surgeons from Austria and an almost perfect score (kappa 0.81/ 95% CI:0.65-0.96) was found between surgeons from France and surgeons from Belgium. Finally, the only moderate interrater agreement (kappa 0.60/ 95% CI:0.42-0.79) was found in comparing the cosmetic evaluation of surgeons from France and surgeons from Austria.

Discussion

The current innovative and popular character of AFT makes it one of the fastest developing surgical techniques in plastic surgery. This trend is noticeable in various different aspects of AFT, such as the technique, its indications and the way we try to increase its results through supplementation or external expansion (BRAVA). While most of these developments are not new, improvements in the way we measure its efficacy and patient satisfaction, have only recently began to evolve. Up until 2011, most studies only superficially mentioned good patient/ surgeon satisfaction with only a few using some sort of Likert Scale.

Since then, the value of patient reported outcomes measurements (PROM) has gradually permeated in the world of AFT with several studies reporting patient satisfaction of AFT after breast reconstruction with either study specific PROM's [19] or validated questionnaires like the Breast-Q [15,16,20]. However, the Breast-Q, like other PROM's primarily reports on patient satisfaction and comparisons between the cosmetic evaluation of AFT from patients and surgeons cannot be made. At the same time a quantative objectification of the difference between what the doctor describes as "beautiful" and what the patient's perception thereof is, might actually prove very helpful in the consultation room when discussing expectations preoperatively.

The overall agreement between the surgeons and the group of augmentation-, control group- and DIEP reconstruction patients was fair, moderate and substantial, respectively over all sets of photographs. This indicates that overall, DIEP patients are more likely to share the same cosmetic appreciation as surgeons - when it comes to the use of BRAVA + AFT for various indications. However, augmentation patients only showed a fair interrater agreement with the surgeons and this group might therefore benefit from a more extensive form of preoperative patient education, specifically highlighting the surgeons expectations of the postoperative effect. While the agreement between surgeons and patient groups varied, patients groups amongst each other, for the larger part, shared the same cosmetic evaluation on all BRAVA + AFT indications. In addition, we observed that surgeons from different European countries shared the same cosmetic values. This indicates that patient education, performed by a surgeon from a neighboring country, is not colored by differences in the cosmetic appreciations of the procedure inherited from the native country. The scoring trend only showed very low or even negative interrater agreements between groups. This indicates that while the interrater agreement between groups ranges from substantial to almost perfect *per photograph*, no such agreement could be found between groups, when looking at the increase of cosmetic appreciation. Fortunately, patients tend to be more optimistic regarding the postoperative results, especially DIEP patients compared to surgeons on the indication that mattered most for this group (fig 1/ breast reconstruction after mastectomy). This suggests that there is a chance that the patient is more satisfied with the end-result than what would be expected based on the information provided by the surgeon preoperatively.

Limitations

This study is limited by its design. Most studies report the satisfaction of patients with their own breasts, and this satisfaction might significantly differ from the appreciation of cosmetic results of a procedure based on photographs from another woman. Furthermore, all photographs illustrate the postoperative effect of BRAVA + AFT which is generally better than solitary AFT and are therefore not reproducible for the latter. Finally, patients from group 1 and 2 were studied postoperatively and their cosmetic evaluation of the photographs might have differed when studied preoperatively.

Conclusion

This study illustrates, for the first time, the interrater agreement and scoring trends between European plastic surgeons and different patient groups in the cosmetic evaluation of BRAVA + AFT for various indications in breast surgery. The most quantative similarities were found between surgeons and DIEP patients. However, DIEP patients are generally more appreciative of the cosmetic results of BRAVA + AFT for breast reconstruction after total mastectomy and augmentation patients tend to agree the least with surgeons on all indications. Further studies should focus on the qualitative nature of the differences between surgeon- and patient appreciation with this technique in order for us to increase the quality of patient-surgeon communications. In the meantime it might be beneficial for surgeons to elaborate more on expectations when educating the patient seeking BRAVA + AFT for breast augmentation purposes.

Ethical approval

This study was approved by the medical ethics committee of Maastricht University Medical Center (METC-16-4-167.1/ab)

Funding

None.

Conflict of interest statement

None.

Acknowledgements

None

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Chapter 9

Autologous Fat Transfer for Facial Rejuvenation; A systematic Review on Technique, Efficacy and Satisfaction

Groen JW, Krastev TK, Hommes J, Wilschut JA, Ritt MJPF, van der Hulst RRJW. Plastic and Reconstructive Surgery Global Open, 2017;5:e1606, Published online 22 December 2017

Abstract

Background: Parallel to the steady decline in surgical aesthetic procedures to the face, dermal-fillers seem to gain a more prominent place in facial-rejuvenation over the last couple of years. As a dermal, facial-filler, autologous-fat-transfer (AFT) seems to have real potential because of the biocompatibility of adipose tissue besides being a procedure with few and primarily minor complications. This systematic-review aims to evaluate the available evidence regarding the safety and effectiveness of AFT for facial-rejuvenation.

Method: A systematic-review following the Preferred-Reporting-Items-for-Systematic-Reviews-and-Meta-Analysis (PRISMA) statement was conducted. MEDLINE, Embase and Cochrane-Library were searched up to December 2016, with no language restrictions imposed. Case-series, cohort studies and randomized-controlled-trials (RCTs) reporting on relevant outcomes were included.

Results: Eighteen clinical articles were included reporting on 3,073 patients in total over a mean follow-up period of 13.9 months. Meta-analysis showed an overall complication rate of 6% (95% CI 3.0-14.0), with hematoma/ ecchymosis (5%), fat necrosis/ oil cysts (2%), irregular fat distribution and scars (both 2%) being amongst the most reported. No major complications were reported and the overall patient-satisfaction rate was 81%.

Conclusion: Although the evidence in this systematic-review is still limited and plagued by heterogeneity between studies, AFT seems to be a promising method in facial rejuvenation with fewer complications than other fillers and high patient satisfaction rates. Further large-cohort, preferably multicenter, RCTs should substantiate these results through quantifiable volumetric-assessment-tools and validated patient-questionnaires, while adhering to predetermined nomenclature in terms of complications.

Introduction

For ages the face has been considered the most prominent feature of the human being and the motivation to alter its appearance for cosmetic purposes is as old as the work of Sushruta [1]. Over the past decades, fueled by western media adjusting to the growing older population, there has been an increasing demand for minimally invasive cosmetic procedures that enhance or maintain the youthful-looking appearance of the face [2]. The 17% decrease of facial surgical cosmetic procedures since 2000 [3,4] combined with the 6.5% increase of Hyaluronic-Acid, globally in 2015 [5] further illustrates the growing demand for dermal-fillers. The ideal filler opposes much of the aspects that menace the aging face (sagging, skin-atrophy), while at the same time being predictable, adjustable to facial anatomy and especially biocompatible [6]. None of the numerous soft tissue augmentation products currently approved by the FDA, both temporary fillers as well as permanent fillers adhere perfectly to these qualities and complications range from minor (bruising) to severe (embolisms, blindness) [7,8]. As a result, it was not long before Autologous Fat Transfer (AFT) or lipofilling found its way as a potentially superior facial filler with numerous studies reporting on the promising results besides minimal side effects [9-11]. Numerous reviews and articles describing the authors preferred method for facial AFT currently exist [12-26], but they generally lack a comprehensive study-design. Furthermore, the abundance of anatomical facial-zones further complicates pooling of data, with most authors describing its appliance to one or two facial regions [10]. Therefore, the aim of this systematic-review was to determine the rejuvenating properties of AFT to the whole face in terms of volume enhancement and patient/surgeon satisfaction and objectify these terms by determining technique, complications, volume-retention and specific patient/surgeon satisfaction rates.

Methods

A systematic-review of literature reporting on technique, efficacy and patient/surgeon satisfaction rates regarding AFT for facial rejuvenation, was conducted according to the preferred-reporting-items-for-systematic-reviews-and-meta-analysis (PRISMA) statement [27]. Medline (Ovid), Embase.com and Cochrane-Library (Wiley) were searched from inception (by JG and TK) up to December 11, 2016. The following terms were used (including synonyms and closely related words) as index terms or free-text words: 'facial' and 'rejuvena-

tion' or 'aging'or 'wrinkles' and 'Autologous-Fat-Transfer'. The full search strategies can be found in the Supplementary Information (Appendix S1). Studies that were considered relevant based on the titles were stored using Endnote® [28], with no restriction on language, study-design or publication media. Bibliographies of relevant articles were manually searched for relevant or missed references.

Eligibility criteria

Original randomized-controlled-trials (RCT's) and cohort-studies on facial rejuvenation with the use of AFT with or without supplementation, which reported on efficacy (i.e. volume enhancement, improving skin trophicity and decreasing wrinkles), technique and patient/surgeon satisfaction were included. Studies reporting on AFT for facial rejuvenation in conjunction with/ or following other surgical procedures or injectables were excluded. However, studies combining AFT with laser-resurfacing techniques or studies that included combinations of treatment (i.e. AFT + surgical procedures) but clearly reported on AFT specific complications were included. Duplicate articles, case-reports or case-series with a sample size <10 and articles with a mean follow-up period <6 months were excluded.

Study selection

Articles were screened for relevancy by two independent reviewers (JG, TK). When considered eligible by both reviewers the full-text article was retrieved for possible inclusion. Discrepancies between the two reviewers were discussed and when a solution was not found a third reviewer (JH) was consulted. When a study could not be retrieved, the authors were contacted to request a copy of the original article.

Outcome measures

We included the following outcomes:

1) Facial rejuvenating properties (i.e. volume enhancement, improving skin trophicity, decreasing wrinkles) objectified in numerical (i.e. percentile) or ordinal scale.
2) Complications
3) Patient/surgeon satisfaction

Data extraction

Data was extracted by one researcher (JG) using standardized tables developed for this purpose and checked by a second reviewer (TK). Extracted data included: country, publication year, study-design, number of subjects, AFT technique, complication rate and management, volumetric measurements and satisfaction rates. Included studies were evaluated with respect to the following factors: inclusion/ exclusion criteria, patient selection (i.e. consecutive versus non-consecutive recruitment) and use of objective outcomes. Included studies were assigned a level of evidence (OCEBM, 2011) by two independent reviewers (JG, TK). The principal summary measures are rates or actual numbers with percentages given between parentheses, besides means over follow-up periods.

Assesment of risk of bias

Observational studies and clinical trials without detailed randomization protocols were considered studies with high risk of bias. The-Cochrane-Risk-of-Bias-for-Randomized-Clinical-Trials [29] and Risk-Of-Bias-In-Non-randomized-Studies-of-Interventions (ROBINS-I) [30] were used for quantifying the risk of bias across RCT's and non-RCT's respectively.

Data synthesis

In accordance with the Cochrane-Handbook-for-Meta-analyses, in the studies that compared two methods only the data from the group treated with AFT was used [31].

Statistical analysis

R statistical software was used for analyzing the data [32]. The pooled proportion of complications was estimated by both a fixed and random effects model. The amount of heterogeneity between the studies was tested with Cochrane's Q and quantified with I^2. A random effects model was used if Q was significant, a fixed effects model otherwise [33,34].

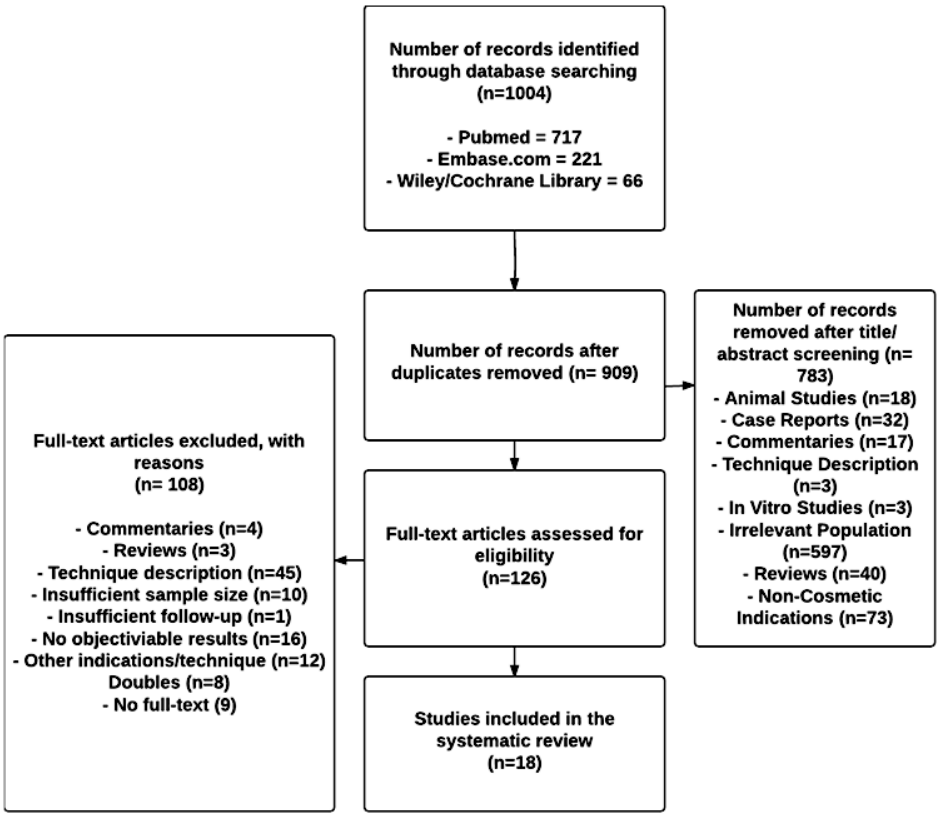

Figure 1: Flow diagram of selected studies

Results

There was a high inter-rater-agreement, in selecting relevant articles based on the abstract screening, of 0.88. After screening (Figure 1), a total of 18 – English written – articles were included. The risk of bias across the cohort studies (Table 1) was considered moderate in 80%. The risk of bias of the three comparative studies is illustrated in figure 2. Extracted data are summarized in tables 1-5. The included studies were published between 1990 and 2016, with 13 retrospective and 2 prospective cohort designs next to 3 trials. There were 17 level-III studies and one level-II study involving a total of 3,073 patients. Two studies [35,36] studied the same set of patients by applying different methods of preparation or supplementation respectively using two different sides of the face (split over a vertical axis). The mean follow-up period was 13.9 months (range 9-133 months).

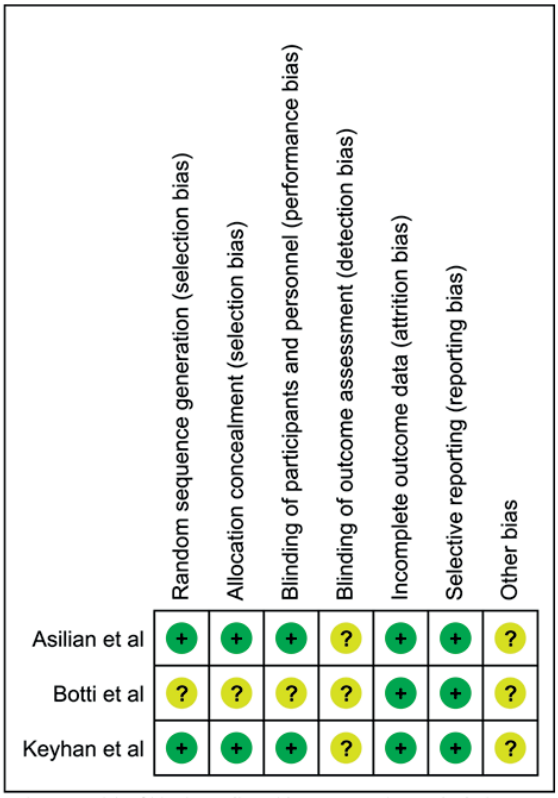

Figure 2: Risk of Bias in comparative studies

Table 1: Baseline: Characteristics of included Studies

Reference	Year	Country	Study-design	Number of patients	Female patients (%)	Exclusion Criteria	Age (years): mean/ median (SD/ range)	Reported outcomes	Follow up (months): mean/ median (SD/ range)	Level of Evidence (OCEBM [68])	ROBINS-I
Gormley et al. [40]	1990	US	Retrospective cohort study	18	18 (100%)	-	r 35-70	FTG, VA	12 (actual)	III	Moderate
Eremia et al. [39]	2000	US	Retrospective cohort study	116	115 (99.1%)	- Less than two AFG treatments - Less than 12 months follow up	57 (r 34-72)	FTG, C, S	r 9-14	III	Moderate
Dasiou et al. [38]	2004	Greece	Retrospective cohort study	1720	-	-	-	FTG, VA	r 12-24	III	Moderate
Botti et al.[35]	2010	Italy/ Romania	Prospective double-blind cohort study	25 (BI)	21 (84%)	- Chemotherapy - Radiotherapy - Chronic steroid use - Connective tissue disease - Chronic blood abnormalities - Systemic metabolic disorders - History of obesity - Body Dismorphic disorder - Anticoagulant treatment, - Assymetric facial features	46.3 (r 21-72)	FTG, S	6 (actual)	III	/
Xie et al. [45]	2010	China/ US	Retrospective cohort study	83	64 (77.1%)	- Absence of clear indication (i.e. temporal-, cheek-, periocular hollowing, lean or aging face, facial asymmetry)	53.16 (range 18-55)	FTG, C, S	32 (mean)	III	Moderate
Monreal et al. [48]	2011	Spain	Retrospective cohort study	18	-	-	-	FTG, C	12 (actual)	III	Serious
Ransom et al. [49]	2011	US	Retrospective cohort study	17	17 (100%)	- Incomplete photographical documentation	61 (range 49-74)	FTG, C	7 (mean)	III	Moderate

Reference	Year	Country	Study-design	Number of patients	Female patients (%)	Exclusion Criteria	Age (years): mean/ median (SD/ range)	Reported outcomes	Follow up (months): mean/ median (SD/ range)	Level of Evidence (OCEBM [68])	ROBINS-I
Tsai et al. [50]	2011	Taiwan	Retrospective cohort study	209	-	- Satisfaction with NLF - History of previous easthetic facial surgery - Previous use of fillers	46.7 (r 35-61)	FTG, C, S	24 (actual)	III	Moderate
Li et al. [42]	2012	China	Retrospective cohort study	38	38 (100%)	-	29.5 (SD 6.8)	FTG, C, VA, S	6 (actual)	III	Moderate
Scorza et al. [43]	2012	Italy	Retrospective cohort study	215	215 (100%)	- Current anticoagulant treatment - Pregnancy - Previous use of fillers - Bacterial/ fungal/ viral skin infection - Systemic disease (diabetes, coagulation disorders, connective tissue disease) - Aberrant laboratory values: (CBC, liver/ kidney function, elektrolytes, coagulation profile, serology for Hepatitis and HIV) - Cardiac disease - Male sex - Age <45, >70	55.5 (SD 2.1)	FTG, C, S	12 (actual)	III	Moderate
Zeltzer et al. [51]	2012	Belgium	Retrospective cohort study	250	222 (89%)	-	53 (r 35-71)	FTG, C	14 (mean), r 1-36	III	Moderate

Reference	Year	Country	Study-design	Number of patients	Female patients (%)	Exclusion Criteria	Age (years): mean/ median (SD/ range)	Reported outcomes	Follow up (months): mean/ median (SD/ range)	Level of Evidence (OCEBM [68])	ROBINS-I
Keyhan et al. [36]	2013	Iran	Double-blind prospective clinical trial	25 (BI)	17 (68%)	- Previous or additional surgical procedures - Compromising systemic conditions (platelet dysfunction, thrombocytopenia) - Hemoglobin <10g/dl) - Infection - History of local or systemic corticosteroid consumption - Addiction - Dramatic weight gain/ loss in previous month	45 (r 24-69)	FTG, C, VA, S	12 (actual)	II	/
Asilian et al.[37]	2014	Iran	Prospective comparative cohort study	32	-	- Severe photo-aging - Coagulopathy disorder - Severe systemic disease - Infection - Previous fat or gel injection at nasolabial fold	-	FTG, S	12 (actual)	III	/
Le et al. [47]	2014	US	Retrospective cohort study	70	65 (93%)	- Prior eyelid surgery	53	FTG, C	10 (actual)	III	Moderate
Bernardini et al. [46]	2015	Italy	Retrospective cohort study	98	92 (94%)	-	51 (r 27-74)	FTG, C, VA	6 (mean)	III	Moderate
Schendel et al. [44]	2015	US	Prospective cohort study	10	10 (100%)	- Insufficient 3D scan - Large weight fluctuations - Additional facial surgery	51.6 (SD 9.57, range 36-71)	FTG, VA	12.6 (mean)	III	Moderate
Ibrahiem et al. [41]	2016	Egypt	Retrospective cohort study	(66/104 for facial AFG)	-	-	34	FTG, C,S	39 (r 12-133)	III	Serious

Reference	Year	Country	Study-design	Number of patients	Female patients (%)	Exclusion Criteria	Age (years): mean/ median (SD/ range)	Reported outcomes	Follow up (months): mean/ median (SD/ range)	Level of Evidence (OCEBM [68])	ROBINS-I
Tepavcevic et al. [9]	2016	Serbia	Prospective cohort study	63	56 (88.9%)	- Preexisting psychiatric disorder - Antidepressive therapy during last month	50.0 (SD 9.6, range 29-68)	FTG, S	6 (actual)	III	Serious

Abbreviations: Not Reported (-), Not specified (NS), Fat Grafting Technique (FTG), Complications (C), Volumetric Assessment (VA), Satisfaction (S), Range (r), Bilateral injection (BI*[1)], The-Cochrane-Risk-of-Bias-for-Randomized-Clinical-Trials (CRoB-RCT), Nasolabial Fold (NLF),

*[1]: Study-designs in which the face was divided through the horizontal axis and both sides were used for different treatment methods (i.e. different preparation, with or without supplementation etc)

Fat grafting technique

All articles described, to some extent, the methods of preparing and grafting the adipose tissue (Table 2) [9,35-51]. Eleven out of 14 studies used a local form of anesthesia [9,36-45,47,48,50] and three authors preferred general anesthesia [35,46,51]. The abdomen was the primary donor-site in most studies with fat from the thigh and flank area used in cases of insufficient supply. The infiltration cannula-size was poorly reported, with three studies [35,38,43] reporting using 1, 2, or 3 mm cannulas respectively and the infiltration solution varied widely amongst studies. Ten studies [9,35,37,38,42,43,45-47,51] (additionally) used some form of local anesthetic in combination with different solutions of epinephrine and saline before harvesting by way of manual-aspiration in 16 out of the 18 reporting studies. Harvesting was done by 2-3mm cannulas, mostly blunt with 2-3 holes and attached to 10-60 cc Luer-Lock-Syringes. Preparation of the adipose tissue was done solely by centrifugation in 5 studies [9,36,42,46,50] ranging from 1,000-3,000rpm over 1-3 minutes spans, with the studies of Asilian et al. [37] and Botti et al. [35] comparing centrifugation and washing between groups. Furthermore, 6 studies [38-40,44,45,49] used combinations of preparations in a none-comparative study-design. Stromal-Vascular-Fraction (SVF), Platelet-Rich-Fibrin (PRF) and Platelet-Rich-Plasma (PRP) were used to supplement the fat in four studies, two by comparative design [36,42]. The injection cannula-sizes ranged from 1-3mm (14-23 Gauge) and were mostly blunt with two studies reporting using lateral openings [35,37] and one study using a ratchet gun for precise fat-distribution [43]. For the injections, most studies described a retrograde injection-technique. The primary site of injection was the subcutaneous space with additional injections most often performed above or just beneath the superficial-muscular-aponeurotic-system (SMAS). The number of AFT sessions was reported in 11 studies [9,36,38,39,41-45,47,51] and varied from 1-4 with an mean interval of 4.25 months [38,39,41,45,47,51]. Postoperative management varied greatly amongst the 9 reporting studies [35,37,39,41-43,45,49,50] and was even contradictory with Ibrahiem et al. [41] recommending massage, as opposed to other studies.

Table 2: Fat grafting technique: Overview of the form of anaesthesia, donor site, infiltration solution, harvesting-, preparation-, and injection technique used. Addition of supplementation, the number of sessions and the injected volume are subsequently given.

Reference	Year	# pat.	Anesth.	Donor Site	Infiltration cannula	Infiltration solution	Harv. meth.	Harvesting cannula + Syringe	Preparation	Supp	Inj. cann.	Inj. Techn.	Inj. Planes	No. sessions (n)	Postop care	Other
Gormley et al. [40]	1990	18	Loc	Abd	-	Lido 1% (skin) + NaCl (c) (subcut)	MA	14 G + 10ml LLS	NaCl washing + Decantation (NS)	-	14 G	-	Intrad	-	-	oc (NS)
Eremia et al. [39]	2000	116	-	Abd, Th, Fl	-	NaCl (c)	MA	14 G + 20-30ml LLS	NaCl washing + Decantation*[1] (NS)	-	14 G (16 G glabella region)	Rg (+ fat-molding for lips)	Subcut	2 (3 in 52/116)	Syst AB (6d postop)	oc (NS)
Dasiou et al. [38]	2004	1720	Loc (+ diazepam when indicated)	Abd, Th, Kn, Gm, Tr, Fl	2mm	Lido 0.1% + epi 1:10^5 + NaBic 20mEq/l	MA	2-3mm/ bl + 20ml LLS	Dec (x2) + C: 2000rpm/ 2 min	-	21-23 G	Rg/ Fan	Subcut, intrad	Multi. (NS)	-	FF (-30ºC/ 24m)
Botti et al.[35]	2010	- 25 (r) - 25 (l)	Gen (Sed)	Abd, Kn, Th	3mm (Klein)	NaCl +0.25% mepi + epi 1:5x10^5	MA	2mm/ bl/ 2h + 10ml LLS	- C: 3000rpm/ 3 min - Sterile filtering + NaCl washing	-	1-2mm/ bl/ lo	Rg/ Fan	Multi (NS)	-	Steri inj-s, syst AB, c/ compr dress donor-s/ inj-s	-
Xie et al. [45]	2010	83	Loc	Abd, Th	-	Lido 0.08% + Epi 1:5x10^5	MA	2.5mm/ 2h + 60ml LLS	Intra-syringe NaCl washing + C: 1000rpm/ 2 min	-	2-3mm/ bl	Rg/ Fan	Subcut, sub-SMAS	1-3 (NS)	Compr dress (1w), facial inactivity instr	oc (20-30%)
Monreal et al. [48]	2011	18	Loc	Abd, Th	-	-	MA	3mm/ multi-h + 10ml syringe (NS)	Dec: 20 min	-	1.2-1.4 mm/bl	Rg	Subcut, SMAS	-	-	-
Ransom et al. [49]	2011	17	-	-	Coleman (NS)	Coleman (NS)	MA	Coleman cannula NS + 10 cc LLS	Dec 10 min + C 3000rpm/ 3 min	-	17 G	NS	-	-	Gentle cleansing + Aquaphor® ointment for 1 week, avoidance of sunexposure	AFT + Laser (CO_2) resurfacing
Tsai et al. [50]	2011	209	Loc	- (pp)	Coleman (NS)	Coleman (NS)	MA	-	C (NS)	-	NS (3 ml syringe)	-	NLF (NS)	-	Mass contr-i, facial activity	Dissection of NLF - DFA

Reference	Year	# pat.	Anesth.	Donor Site	Infiltration cannula	Infiltration solution	Harv. meth.	Harvesting cannula + Syringe	Preparation	Supp	Inj. cann.	Inj. Techn.	Inj. Planes	No. sessions (n)	Postop care	Other
Li et al. [42]	2012	- 26 - 12	Loc	Abd, Th	-	Lido 0.08% + epi 1:5x10^5	MA	2.5mm/ 2h	C: 1000rpm/ 3 min	- SVF - None	1,5/ 3mm	Rg/ Fan	Subcut, sub-SMAS	1	Compr dress, facial inactivity instr	oc (20-30%)
Scorza et al. [43]	2012	215	Loc	Abd, Tr, Th, Kn	1mm	NaCl 500ml + 25ml lido 1% + epi 0.5ml + tri/ac 40 mg/ml + NaBic 2ml	MA	2mm/ bl + 10ml LLS	10-12 Cycles of intra-syringe washing	-	17 G/ bl (+ Ratchet Gun)	Rg/ 0,1ml/cm3 through Ratchet Gun)	Subcut, above SMAS	2	Steri inj-s, AB cream	-
Zeltzer et al. [51]	2012	250	Gen/ Loc (NS)	Abd, Fl, Th, Kn	-	mod Klein (800 mg lido + epi 1/1x10^6)	MA/ LD*[2]	2-3mm/ bl/ 10h + 10ml LLS	Washing (NS)	-	23 G/ sh	Rg	Intrad	1 (218), 2 (32)	-	-
Keyhan et al. [36]	2013	- 25 - 25	-	Kn, Abd	Coleman (NS)	CS	MA	3mm/ 2h	C: 3000rpm/ 3 min	- PRP - PRF	Var.	Rg	Multi (NS)	1	-	-
Asilian et al.[37]	2014	- 16 - 16	Loc	Fl	-	Lido 0.05% + epi 1:10^6 in LRS	MA	2mm/ bl/ 3h + 10ml LLS	- C: 3400rpm/ 1 min - Sterile filtering + NaCl washing	-	1-1.5mm/ bl/ lo	Rg/ Fan	Subcut	-	Steri inj-s, syst AB, c/ compr dress donor-s, mass contra-i	-
Le et al. [47]	2014	70	Loc	Kn, Th	-	Lido 1% + Epi 1:1x10^5 + NaCl	-	14 G/ bl	Washing (NS)	-	0.9-1.2mm/ bl	Fan	Orb. musc./ mal. fatpad	1 (54), 2 (15), 3 (1)	-	-
Bernardini et al. [46]	2015	98	Gen (Sed)	Sp, Tr, Kn	-	LRS (500ml) + lido (500mg) + NaBic 5 mEq + Epi 0.5%	MA	2mm/ multi-h	C: 2000rpm/ 1 min	PRP	20-23 G/ sh	-	Subcut, musc	-	-	-
Schendel et al. [44]	2015	10	-	-	-	-	LD	3mm/ 2h	C + washing (multi. sessions, NS)	SVF	CC (NS)	-	NS (surgeons discretion)	1	-	-
Ibrahiem et al. [41]	2016	66	Loc (Gen when indicated)	Abd, Th	BLI	LRS (500ml) + ligno 2% 20ml + epi 0.5ml 1:2x10^5	MA	2.1mm/ bl +60ml LLS	Decantation (30 min)	-	-	-	-	3-4 (NS)	w/ compr dress, mass of inj-s, loc AB	FF (-18 ºC)

Reference	Year	# pat.	Anesth.	Donor Site	Infiltration cannula	Infiltration solution	Harv. meth.	Harvesting cannula + Syringe	Preparation	Supp	Inj. cann.	Inj. Techn.	Inj. Planes	No. sessions (n)	Postop care	Other
Tepavcevic et al. [9]	2016	63	Loc	Abd, Tr	-	Lido 0.5ml + Epi MA 1:2x10^5		3mm/ 3h + 10ml LLS	C: 3000rpm/ 3 min	-	-	-	-	1	-	-

Abbreviations: Autologous Fat Transfer (AFT), Abdomen (Abd), Analgesic Sedation (Sed), Antibiotics (AB), Blunt (bl), Blunt Lamis Infiltrator® (BLI), Cannula (cann), Centrifugation (C), Cold (c), Coleman Cannula (CC), Coleman's Solution (CS), Compressing (compr), Contra-indicated (contra-i), Day's (d), Decantation (dec), Donorsite (donor-s), Dressings (dress), Epinephrine (epi.), Fanning (Fan), Flanks (Fl), Freezing of Fat (FF), Gauge (G), General Anaesthesia (Gen), Gluteus region (Gm), Harvesting Method (Harv. Meth.), Injection (inj.), Injection-site (inj-s), Instructions (instr), Intradermal (intrad), Knees (Kn), Lactate Ringer Solution (LRS), Lateral opening (lo), Lidocaine (lido), Lignocaine (ligno), Liposuction Device (LD), Liter (l), Local Anaesthesia (Loc), Luer Lock Syringe (LLS), Manual Aspiration (MA), Massage (mass), Mepivacaine (mepi), Milliequivalent (mEq), Minute (min), Months (m), Multiple (multi), Not Reported (-), Not Specified (NS), Number of cannula holes (#h), Overcorrection (oc), Platelet-rich-Fibrin (PRF), Platelet-rich-plasma (PRP), Postoperative (postop), Range per minute (rpm), Retrograde (Rg), Right (r), Left (l), Sodium-Bicarbonate (NaBic), Sodium-Chloride (NaCl), Steristrips (steri), Stromal Vascular Fraction (SVF), Subcutaneous (subcut), Superficial Muscular Aponeurotic System (SMAS), Systemic (syst), Technique (techn), Thighs (Th), Triamcinolone Acetonide (tri/ac), Trochanteric region (Tr), Under (sub), Variable (var), Warm (w), Week (w), Orbicularis (Orb), Muscle (musc), Malar (mal) Sample size (n) patients preference (pp), Nasolabial Fold (NLF), Derma-fascial Attachement (DFA), Modified (mod), Sharp (sh), Suprapubic (Sp),

*[1]: Sedimantation (the process of settling down of heavy solids in a mixture of a liquid and insoluble solid) and Decantation (the removal of the clear layer of the liquid without disturbing the settled solids) is used interchangeable since it both describes the process of letting the lipo-aspirate settle in order to remove the desired layer. In the table Decantation is used.

*[2]: Manual aspiration was used when the desired volume of fat was < 10 ml and a liposuction device was used when this exceeded 10 ml.

*[3] Authors report fat grafting in a linear and crosshatching method.

Complications

Meta-analysis was performed over the 12 reporting studies [36,39,41-43,45-51]. To determine the amount of heterogeneity between studies Cochran's Q was calculated (101.45, p< 0.0001) and quantified with I^2 (tau^2 =2.0747;H=3.81[2.98;4.87]; I^2=93.1%[88.7%;95.8%]). According to the Cochrane's Handbook for Systematic-reviews of Interventions [52] – in the case of between trial heterogeneity - the random-effects meta-analysis weights the studies relatively more equally and is therefore used in the following description. The overall complication-rate was 6% (95% CI 3.0-14.0) after a mean follow-up of 15.8 months in 1205 patients (Figure 3/ Table 3). Hematoma/ ecchymosis was most reported (5%, 95% CI 2.0-15.0), followed by fat necrosis/ oil cysts (2%, 95% CI 1.0-5.0), irregular fat distribution and scars (both 2%, 95% CI 1.0-4.0). Infections were reported in 1% (95% CI 0.0-4.0) of 728 patients in six studies.

Table 3: Complications: Overview of complications and management

Study	Year	Pat#	Complications (%)	Management
Eremia et al. [39]	2000	116	- Infection NR - Hematoma/ ecchymosis 3.3% - Scars 0.9% - Temporary asymmetry 0.9%	- Scars were revised during a subsequent treatment session
Xie et al. [45]	2010	83	- Scars NR - Irregular fat distribution NR	NR
Monreal et al. [48]	2011	18	- Irregular fat distribution NR	
Ransom et al. [49]	2011	17	- Infection NR - Hematoma/ ecchymosis NR - Scars NR - Complete fat resorption (5.9%)	- Hyaluronic acid filler
Tsai et al. [50]	2011	209	Donorsite: - Infection NR - Edema NR - Hematoma/ ecchymosis NR - Irregular fat distribution NR - Scars NR Implantsite: - Infection NR - Edema NR - Hematoma/ ecchymosis NR - Irregular fat distribution NR - Scars NR	NR
Li et al. [42]	2012	38	- Scars NR	NR

Study	Year	Pat#	Complications (%)	Management
Scorza et al. [43]	2012	215	Donorsite: - Bleeding 1.9% - Hematoma/ ecchymosis 0.5% - Hyperpigmented acces points 2.3% - Pain 5.1% Implantsite: - Hematoma/ ecchymosis 7.4% - Fat necrosis/oil cysts 1% - Irregular fat distribution 4.6%	NR
Zeltzer et al. [51]	2012	250	- Edema 9% - Fat necrosis/oil cysts NR - Infection NR - Fat Emboli NR - Hematoma/ ecchymosis 38%	NR
Keyhan et al. [36]	2013	25	- Edema NR - Hematoma/ ecchymosis NR - (Severe) pain NR	NR
Le et al. [47]	2014	70	- Edema 7.0% - Infection NR - Hematoma/ ecchymosis NR - Seroma NR	- Steroid injections (4 out o5 5 patients)
Bernardini et al. [46]	2015	98	- Fat necrosis/oil cysts 3.1% - Irregular fat distribution 1.0%	- Aspiration or surgical removal - NR
Ibrahiem et al. [41]	2016	66	- Infection 6% - Hematoma/ ecchymosis 4.5% (infra-orbital n=2, nasolabial fold n=1) - Fat necrosis/oil cysts 4.5% (all inner-infra-orbital/ upper nasolabial fold)	- NS - Conservative treatment with hot compresses and local heparin crème - Fine needle aspiration

Abbreviations: Not Reported (NR)

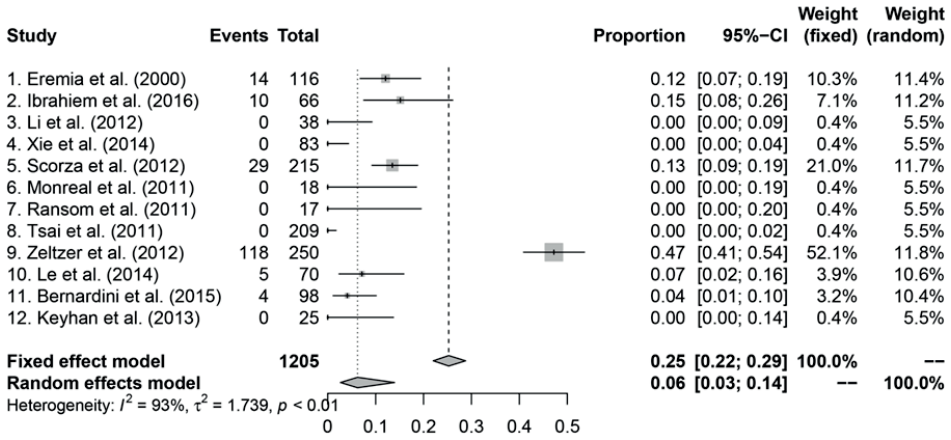

Figure 3a: Overall complications

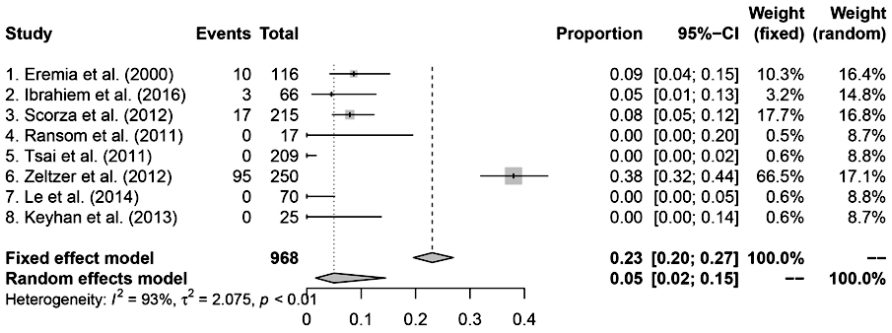

Figure 3b: Hematoma/ Ecchymosis

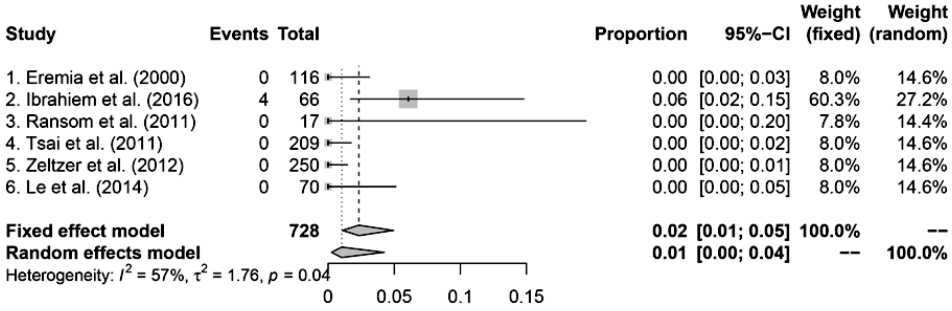

Figure 3c: Infections

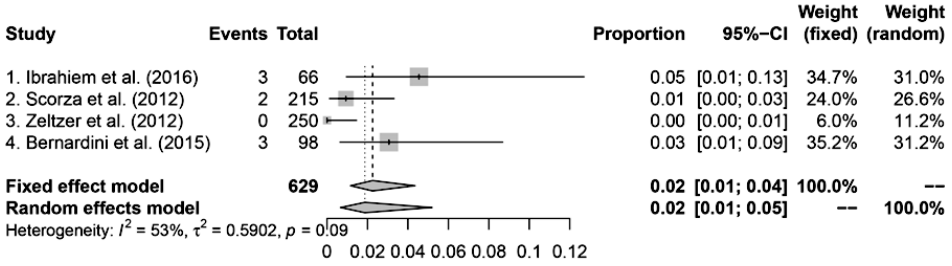

Figure 3d: Fat necrosis/ Oil Cysts

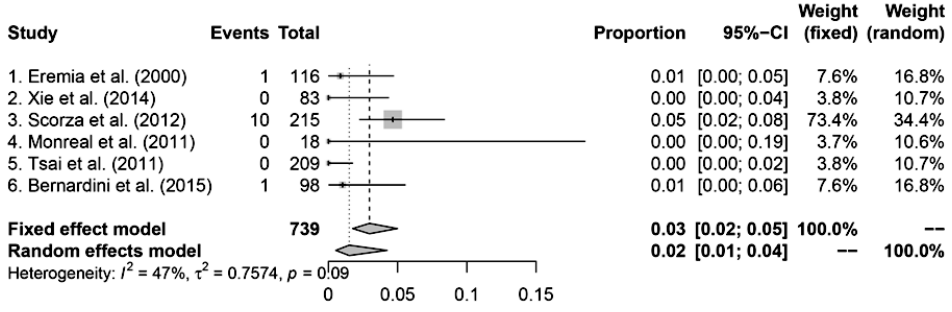

Figure 3e: Irregular Fat Distribution

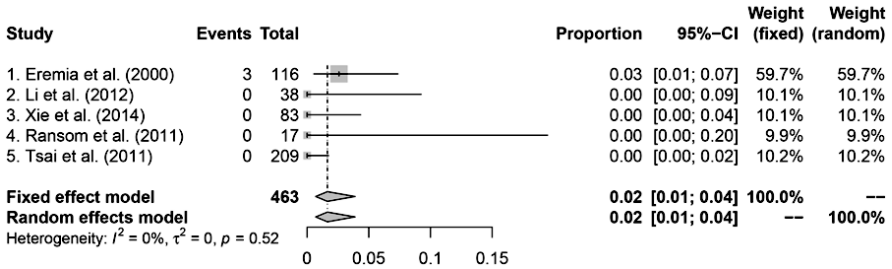

Figure 3f: Scars

Volume retention

Objective measurements of the volumetric result are imperative to demonstrate the efficacy of AFT. However, the face consists of multiple anatomical units greatly varying in important features like density causing great heterogeneity in comparing results. Five studies [36,38,40,42,44] were included in the volumetric analysis (Table 4). The methods of determining volume-retention varied greatly between studies. Supplements added to the fat graft were reported in three studies. As great heterogeneity between studies in regard to injection site and volumetric assessment exists, no pooling of data could be achieved and volume-retention varied greatly from 13-68% over a mean of 12.2 months.

Table 4: Injected volume per facial region and retention: Overview of the auxiliary method, the method of measuring volume retention, the mean total injected volume, the volume gain and the percentage of gain relative to the injected volume.

Study	No. of pat.	Auxiliary method	Meth. of measuring	Inj. vol: Peri-orbital (mean, SD, range)	Inj. vol: NLF (mean, SD, range)	Inj. vol: Forhead (mean, SD, range)	Inj. vol: Lips (mean, SD, range)	Inj. vol: Chin (mean, SD, range)	Inj. vol: Mandible (mean, SD, range)	Inj. vol: Cheek (mean, SD, range)	Inj. vol: Malar (mean, SD, range)	Inj. vol: Marionette Fold (mean, SD, range)	Inj. vol: Glabella (mean, SD, range)	Total inj. vol. (mean, SD, range)	Volume gain relative to graft volume (%)	Follow-up: months (mean, range, actual)
Gormley et al. [40]	18	-	Optical profilometric technique	-	m 2.5 ml	-	-	-	-	-	-	m 1.0 ml	-	m 3.5 ml	19.4%	12 (actual)
Eremia et al. [39]	116	-	-	-	m 2.2 ml (r 1.5-2.5 ml)	-	m 3.7 ml (r 1-2.5 ml)	-	-	-	-	m 1.3 ml (r 1-1.5 ml)	m 1.4 ml (r 1-2 ml)	m 8.9 ml (r 5.5-10 ml)	-	r 9-14
Dasiou et al. [38]	1720	-	Pre-/postop photograph estimation	-	-	-	-	-	-	-	-	-	-	r 3-105 ml	40-60%	r 12-24
Botti et al.[35]	25 (r) 25 (l)	-	-	r 1.5-4 ml	r 2-3 ml	r 2-4 ml	r 3-5 ml	r 2-4 ml	r 4-6 ml	r 5-7 ml	r 3-4 ml	r 3-5 ml	-	r 25.5-42 ml	-	6 (actual)
Xie et al. [45]	83	-	-	m 1.2 ml	-	m 16.5 ml	-	m 2.0 ml	m 11.0 ml	m 20.0 ml	m 7.5 ml	-	-	m 58.2 ml	-	m 32
Li et al. [42]	26	SVF	Pre-/ postop CT + photo comparison	-	-	-	-	-	-	-	-	-	-	- m 17.5 ml (SD 7.3)	- 64.8%	6 (actual)
	12	None												- m 16.2 ml (SD 6.3)	- 46.4%	
Scorza et al. [43]	215	-	-	NS	NS	NS	NS	-	NS	NS	NS	NS	-	m 13.25 ml (SD 3.12)	-	12 (actual)
Keyhan et al. [36]	25	PRP	Pre-/postop photograph analysis in mm	-	-	-	-	-	-	- m 7.0 ml	- m 8.0 ml	-	-	m 15.0 ml	18%	12 (actual)
	25	PRF								- m 7.0 ml	- m 8.0 ml			m 15.0 ml	13%	
Le et al. [47]	70	-	-	0-2 ml	NS	-	-	-	-	NS	NS	NS	NS	5-42 ml	-	10 (actual)
Bernardini et al. [46]	98	-	-	m 3.7 ml	-	-	m 4.9 ml	m 3.5 ml	-	-	m 7.0 ml	-	m 3.0 ml	m 71.4 ml	-	m 6 (r 4-12)
Schendel et al. [44]	10	SVF	Pre-/ postop photocomparison; 3dMD system + Vultus software	NS	-	NS	NS	NS	-	NS	NS	-	NS	m 18.4 ml (SD 15.34)	68%	m 12.6

Study	No. of pat.	Auxiliary method	Meth. of measuring	Inj. vol: Peri-orbital (mean, SD, range)	Inj. vol: NLF (mean, SD, range)	Inj. vol: Forhead (mean, SD, range)	Inj. vol: Lips (mean, SD, range)	Inj. vol: Chin (mean, SD, range)	Inj. vol: Mandible (mean, SD, range)	Inj. vol: Cheek (mean, SD, range)	Inj. vol: Malar (mean, SD, range)	Inj. vol: Marionette Fold (mean, SD, range)	Inj. vol: Glabella (mean, SD, range)	Total inj. vol. (mean, SD, range)	Volume gain relative to graft volume (%)	Follow-up: months (mean, range, actual)
Ibrahiem et al. [41]	66	-	-	-	-	-	-	-	-	-	-	-	-	m 7.0 ml	-	m 39 (r 12-133)
Tepavcevic et al. [9]	63	-	-	m 4.0 ml	m 4.0 ml	m 4.0 ml	-	m 4.0 ml	m 5.5 ml	m 4.5 ml	M 4.5 ml	-	m 4.0 ml	m 34.5 ml	-	6 (actual)

Abbreviations: Nasolabial Fold (NLF), Not reported (-), Not Specified (NS), Range (r), Mean (m),

Patient/ surgeon satisfaction

A total of 9 studies [9,35-37,39,41,43,45,50] reported on patient and/or surgeon satisfaction either on a visual analog scale (VAS) or a 2-4 point Likert scale (Table 5). Meta-analysis for patient satisfaction was performed after conversion to a dichotomous scale (Figure 4). In order to account for between-trial-heterogeneity (Cochran's Q:35.26-6<0.0001/ I^2:tau^2=0.4391;H=2.42[1.72;3.41]; I^2=83.0%[66.3%; 91.4%]) the random-effect-model was used for reporting patient satisfaction. Furthermore, overall-scores were used only postoperatively, and when satisfaction rates were compared between study groups [37] a mean over the total cohort was calculated. The satisfaction rate over a total cohort of 630 patients in six studies [36,37,41,43,45,50] was 81% (95% CI 70.0-89.0). It should be noted that Asilian et al. [37] compared two groups of patients according to preparation method (centrifugation vs filtering/ washing) and both groups were included in the analysis. Surgeons reported a good cosmetic outcome in 89% and the overall postoperative mean VAS score amongst 88 patients in two reporting studies [9,35] was 79,5.

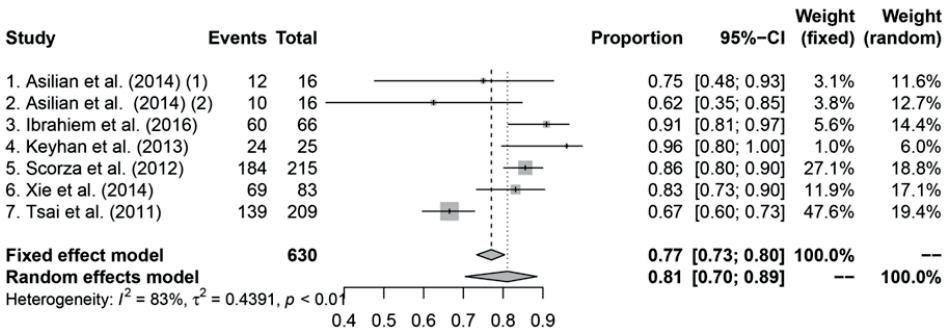

Figure 4: Patients Satisfaction

Table 5: Patient/ Surgeon satisfaction

Study	Year	No. of patients	Follow up (months): mean/ (SD/ range)	Patients satisfaction	Surgeons Satisfaction	
				Three point scale (Satisfied/ Moderately Satisfied/ Dissatisfied)	Measurement:	Three point scale (Good/ Neutral/ Poor)
Eremia et al. [39]	2000	116	r 9-14	-	PPoCc/ TS	Nasolabial Fold (n=85/116): Good 14.1%/ Neutral 21.2%/ Poor 58.8% Glabella (n=26/116): Good 0%/ Neutral 0%/ Poor 100% Lips (n=27/116): Good 0%/ Neutral 0%/ Poor 100%
Xie et al. [45]	2010	83	32 (mean)	Satisfied 83.13%/ Moderately Satisfied 14.46%/ Dissatisfied 2.4%	PPoCc/ IS	Good 86.74%/ Neutral 12.04%/ Poor 1.2%
Tsai et al. [50]	2011	209	24 (actual)	Satisfied 66.7% / Dissatisfied 33.3%	-	-
Scorza et al. [43]	2012	215	12 (actual)	Satisfied 85.6%/ Moderately Satisfied 0%/ Dissatisfied 14.4%	PPoPc/ TS + ID	Good 88.8%/ Neutral 0%/ Poor 11.2%
Keyhan et al. [36]	2013	- 25 - 25	12 (actual)	Satisfied 96%/ Moderately Satisfied 0%/ Dissatisfied 4%	-	-
Asilian et al.[37]	2014	- 16 - 16	12 (actual)	- Satisfied 12.5%/ Moderately Satisfied 62.5%/ Dissatisfied 25% - Satisfied 6.25%/ Moderately Satisfied 56.25%/ Dissatisfied 37.5%	-	-
Ibrahiem et al. [41]	2016	66	39 (r 12-133)	Satisfied 91.0%/ Moderately Satisfied 0%/ Dissatisfied 9.0%	PPoCc/ TS	Good 91.35%/ Neutral 0%/ Poor 8.65%
				Patients satisfaction	Surgeons Satisfaction	
Study	Year	No. of patients	Follow up (months): mean/ (SD/ range)	VAS evaluation 1-10 score (SD)	Measurement:	VAS evaluation 1-10 score (SD)

Study	Year	No. of patients	Follow up (months): mean/ (SD/ range)	Patients satisfaction	Surgeons Satisfaction	
				Three point scale (Satisfied/ Moderately Satisfied/ Dissatisfied)	Measurement:	Three point scale (Good/ Neutral/ Poor)
Botti et al.[35]	2010	- 25 (r/ AFG+C)	6 (actual)	Postoperative score: Temporal 6.7 (1.5), Eyelids 9.1 (1.3), Malar 8.7 (1.3), Tear Through 8.5 (1.5), Cheek 7.0 (2.2), Nasolabial Fold 7.9 (2.0), Lips 6.7 (2.0), Mandible 7.6 (1.8), Marionette Fold 7.6 (2.1), Chin 7.2 (1.7), Global 7.6 (1.9)	PPoPc/ IS + N + MA	Postoperative score: Temporal 6.3 (2.1), Eyelids 9.0 (1.0), Malar 9.0 (1.0), Tear Through 8.7 (1.2), Cheek 6.7 (0.6), Nasolabial Fold 6.7 (0.6), Lips 6.3 (1.5), Mandible 8.7 (1.2), Marionette Fold 7.7 (2.1), Chin 7.0. (1.0), Global 8.0 (1.0)
		- 25 (l/ AFG+W)		Postoperative score: Temporal 6.2 (1.4), Eyelids 8.8 (1.2), Malar 8.9 (1.2), Tear Through 8.5 (1.5), Cheek 7.2 (2.0), Nasolabial Fold 7.6 (2.1), Lips 6.1 (2.0), Mandible 7.4 (1.7), Marionette Fold 7.9 (2.0), Chin 7.4 (1.8), Global 7.5 (1.9)		Postoperative score: Temporal 6.0 (2.0), Eyelids 8.7 (1.2), Malar 9.3 (1.2), Tear Through 8.7 (1.2), Cheek 7.0 (1.0), Nasolabial Fold 6.7 (0.6), Lips 6.3 (1.5), Mandible 7.7 (1.5), Marionette Fold 7.7 (2.0), Chin 7.0 (1.0), Global 7.7 (0.6)
Tepavcevic et al. [9]	2016	63	6 (actual)	Preoperative score: 4.6 (SD1.3) Postoperative score: 8.3 (SD 1.3)	-	-

Abbreviations: Pre-/postoperative photo comparison (PPoPc), Pre/postoperative clinical comparison (PPoCc), Independent Dermatologist (ID), Treating Surgeon(s) (TS), Independent Surgeon (IS), Not Reported (-), Right (r), Left (l), Autologous Fat Grafting (AFG), Centrifugation (C), Saline Washing (W), Nurse (N), Make-up Artist (MA).

Discussion

This study was performed to obtain a comprehensive overview of the available evidence on the outcomes of AFT in facial rejuvenation with objective outcome measures and a clear description of the technique applied. First remarkable issue is the small number of studies to evaluate AFT in rejuvenation of the face. As it is applied numerously over the world; the number of well-designed studies is limited.

As is the case in AFT for other indications – such as the breast – the techniques used for harvesting, preparation and reinjection of the fat varied greatly amongst authors. The most important aim in this continuing search for the golden standard in AFT is improving the volume-retention which is believed to be influenced by almost all the AFT aspects. Whether shear stress of the adipocytes caused by cannula size (either during harvesting or injection) or high osmolality of the infiltration solution play a role remains a matter of debate. Both have been shown to vary greatly in this systematic-review but have also been shown to matter significantly to the long-term volume-retention [53]. Two recently published in vitro studies [54,55] shed some light on this interesting topic with Hivernaud et al. [55] reporting on – amongst others – adipose tissue resorption variances between different combinations of harvesting (i.e. manual, power-assisted or water-assisted lipoaspiration) and preparation (i.e. decantation, centrifugation, or filtration). They found that in both the in-vitro as well as in the murine models greater efficiency (in terms of retaining tissue volume) was achieved with manual aspiration, soft centrifugation (400g for 1 min) and washing steps. While the majority of studies in this systematic-review used manual aspiration, the centrifugation settings and times were considerably higher. Secondly, Streit et al. [54] further studied the differences in morphology between fat samples obtained through decantation, centrifugation, and membrane-based tissue filtration and found the highest numbers of adipose derived stem cells in the upper fraction of centrifuged lipo-aspirates but the maximal concentration of adipose fraction after membrane based tissue filtration. In conclusion, both studies seem to suggest superiority of manual aspiration and centrifugation and/or washing procedures – in line with both the British and German clinical guidelines [56,57] – but longer follow-up for the former and affirmation in clinical practice for the latter study is necessary to make conclusive statements. As was stated in the recent systematic-review of Shim et al. [58], the same can be said for harvest location, since multiple studies have shown a great varying degree in adipocyte number, volume and morphology as well as adipocyte-derived-stem-cells depending on where the fat is harvested.

Complications after dermal-fillers are usually divided in early and late events and again into minor and major [8]. One of the advantages of AFT over other facial fillers in both early and late events is the absence of hypersensitivity reactions and granuloma formation respectively. Furthermore, when comparing AFT with the use of Hyaluronic Acid (HA) fillers, major complications such as necrosis and blindness – which have both been described following HA injection [59-62] – were not reported. The most reported complication following AFT for facial rejuvenation – hematoma/ ecchymosis – was reported in 5% (95% CI 2.0-15.0) of the total cohort which is in line with that reported in studies using other dermal-fillers [63]. Late onset complications such as fat necrosis (2.0%, n=629) have been reported but are amongst the other complications[10,11] minimal.

As stated before, the long-term volume-retention is crucial in defining AFT as a biocompatible permanent filler in general and in verifying its superiority over other fillers. Three studies [38,42,44] reported an overall volume-retention ranging from 40 to 68% over a follow-up of 6 to 12 months without specifying the injected locations. The remaining studies [36,40] while specifying the locations (nasolabial/marionette fold and cheek/malar respectively) reported much lower volume-retentions, ranging from 13 to 19% over a follow-up of 12 months indicating the importance of the location in regard to the long-term retention of the reinjected fat. However, due to the great heterogeneity amongst studies – especially when it comes to the different injected facial zones – no definitive conclusion could be made with regard to overall volume-retention after AFT for facial rejuvenation. Supplements were used in two studies that reported on volume retention [36,44], however the injected facial zones, the method of measuring volume retention and the supplements used (PrP/PrF vs SVF) al varied, so no beneficial effect could be reported. Therefore, the aim of further studies should be towards facial location-specific volumetric assessment using objectifiable tools like 3D imaging (such as the VECTRA XT® 3D imaging system), CT or MRI.

The patient and surgeon satisfaction rates in the included studies were considered acceptable and in line with other publications as well as a recently published study on quality of life after minimally invasive facial cosmetic procedures [64]. However, only standard visual analog scales, as well as Likert scales were used without the inclusion of validated questionnaires like the FACE-Q [65]. Also satisfaction scores per facial zone are only reported in one study [35] on VAS, ranging from 6 in the lips and 9 in the eyelids and malar region. Therefore, further studies should focus on incorporating the FACE-Q into the study-design and report per facial zone.

Limitations

This systematic-review has several limitations. Only low-level evidence studies (OCEBM III) and mainly retrospective studies without a control group were found. The three studies that used a comparative study-design failed to report on some important aspects like allocation concealment and blinding as is illustrated in Figure 2. The use of validated measurement tools to assess patient-reported outcomes is lacking, and objectifiable data on volume-retention are generally absent. Heterogeneity between studies in reported outcomes and nomenclature regarding specific facial zones and complications makes it difficult to draw conclusions. This was partly resolved by combining similar terms under one common nominator (e.g., bruising and ecchymosis), but this may have introduced some bias. More important is the fact that several studies neglected to specify the complications and only sufficed with the annotation that there were none. These studies [66,67] were therefore excluded and this adds further to a possible reporting bias. Finally, the very definition of a complication of AFT in facial rejuvenation is a complicated matter and a clear consensus whether, for example, postoperative pain qualifies as a complication or part of the normal postoperative course is still lacking. A strong example thereof is the 38% rate of hematoma in the study of Zeltzer et al. [51], which deviates significantly from the reported rate in the rest of the studies and while the authors tried to correct for this by using a random effect model, the reader should be cautious in interpreting these results. Therefore, on a methodological basis the focus for further studies should be; first to define complications and second to adhere to this definition when reporting on complications. In reporting on patient/surgeon satisfaction the authors took certain liberties in translating Likert scales to dichotomous (satisfied vs dissatisfied) data by categorizing "moderately satisfied" - in a 3-point Likert scale - under "satisfied", since the patients might answered differently when presented with an actual dichotomous question. This should be kept in mind when interpreting these results.

The aim of this study was to complement the broad database of descriptive reviews and expert opinions on the subject of AFT for facial rejuvenation with the addition of a more comprehensive, systematically reviewed overview of the recent literature, including meta-analysis of complications and satisfaction. The authors believe this systematic-review accomplishes that by the inclusion of structured tables on important outcomes as well as the exclusion of case series and case reports and studies with insufficient follow-up periods.

Conclusions

This systematic-review provides an updated overview of the important outcomes of AFT for facial rejuvenation. Although the evidence in this review is still limited and plagued by the same heterogeneity that is often found in reporting on AFT for other indications. Still, this technique is regarded as a promising method in facial rejuvenation. While AFT has a number of obvious advantages over other dermal-fillers in terms of biocompatibility, such as the absence of hypersensitivity reactions and the risks of granuloma formation, other complications such as fat necrosis have to be taken into account. Furthermore, the great variation in reported volume-retentions in this systematic-review suggests further studies are needed to clarify the facial-unit-specific, long-term preservation of the achieved volume before AFT can rightfully be called a true permanent filler. However, in achieving these goals, proper research should evaluate if AFT is the superior biocompatible next generation facial filler.

Disclosures

None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this manuscript..

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Chapter 10

General Discussion

The aim of this thesis was to gain insight into effectiveness and safety of autologous fat transfer (AFT) for various treatment protocols. In this discussion, I will address our research questions. The results will be placed in a broader perspective and the implications for clinical practice will be discussed in the light of the current scientific literature. First, I will discuss important determinants of outcomes regarding the use of AFT in addition to breast reconstruction and augmentation as well as in the treatment of scars. Second, the oncological safety of AFT following breast reconstruction will be further explored by comparing results from both basic science as well as clinical studies. Thereafter, I will further elaborate on clinical practice and experience – both surgeon's as well as patient's - by discussing the outcomes of our two European survey studies. I will conclude this chapter with discussing the effectiveness and safety of AFT as a promising biocompatible tool for facial rejuvenation.

Aft in addition to breast surgery

The history of AFT has known a rocky start and followed an interesting course over the span of the previous century. From Czerny's attempt to correct a partial mastectomy defect using a lipoma [1], to the 1987 ban [2], followed by the standardisation by Dr Coleman [3] and the 2009 official ASPS statement [4]. Through all these developments, the application of its use in addition to *breast surgery* always gained special interest from the plastic surgery community. The following discussion regarding AFT in breast surgery will encompass both breast reconstruction and breast augmentation, with differentiation between the two highlighted when appropriate.

AFT has proven its value in breast surgery in multiple studies, mostly as a way to complement other techniques or as a final "touch-up" to correct small remaining defects or retracted scars. In addition, ongoing clinical trials are studying the use of AFT as a solitary option for both breast reconstruction and -augmentation, generally in combination with auxiliary methods like external expansion e.g. the breast enhancement and shaping (BRAVA) system [5]. Regardless of the specific goal for which AFT is used, there are a number of important aspects to consider. Besides the oncological safety of transplanting adipocyte derived stem cells (ADSC) and their related hormones into a healthy or previously cancerous environment, the treating surgeon should be aware of possible complications, the consequences for radiological follow-up and differences be-

tween grafting techniques. Furthermore, the understanding of patient-/ surgeon satisfaction as well as volume retentions are important aspects to know and discuss preoperatively with the patient.

Oncological Safety

As was discussed in **Chapter 2** the oncological safety of the procedure after breast conserving therapy (BCT) or skin sparing mastectomy (SSM) seems sufficient, with local and distant recurrence rates being lower than those reported in large cohort studies of patients after SSM with immediate reconstruction and in BCT with subsequent whole-breast-irradiation, without AFT [6,7]. These findings are further substantiated by the recently published systematic review of Waked et al. [8], who observed a loco-regional recurrence (LRR) rate ranging 0-3.9% per year following AFT + mastectomy (MST) or BCT in 18 studies. While these overall clinical results on oncological safety after AFT seem favourable, a closer look at some of the matched control studies and their subgroup analysis shows some questionable results. For example, a subgroup analysis in the study of Kronowitz et al. [9], showed an increased risk of LRR after AFT in women treated with hormonal therapy (1.4% vs. 0.5%, p=0.038) and Petit et al. [10,11], found an increased risk for LRR in a subgroup of patients with intra-epithelial neoplasia, a finding they verified in a matched control group analysis with a 5 year follow-up. In spite of the much reported possibility of cancer *recurrence* following AFT for breast reconstruction, cancer *occurrence* after AFT for breast augmentation is an under-reported subject in clinical studies. However, the fear of an increased risk of breast cancer following AFT breast augmentation still exists, partly fuelled by conflicting reports from basic science studies that report on both the carcinogenic and tumour-suppressive capabilities of ADSCs (see **Chapter 6**). Nonetheless, a statement issued by the American Society of Plastic Surgeons (ASPS) suggested there currently is no scientific evidence proving the increased risk of breast malignancy associated with AFT [12]. However, data from subgroup analysis discussed above and the discrepancies between basic science and clinical studies, justify larger follow-up, multicentre prospective trials focussing on questions regarding AFT related breast cancer recurrence or occurrence.

Complications

As with every new surgical technique, complications from AFT, can arise from a variety of factors such as the learning curve or dexterity in instrument handling

and mainly consist of nodules, superficial infections, fat necrosis, cysts, hematoma and calcifications. However, the overall complication rate in **Chapter 2** seemed low in comparison to those described after reconstruction with implants or myocutaneous flaps. In addition, the total complication rate reported after AFT breast augmentation (**Chapter 3)** was considered equal to that after implant based augmentation [13,14]. Fat necrosis and oil cysts appeared to be two of the most common complications following AFT, which seems in line with other studies [15,16]. Both complications are the consequence of the same principle, which mostly results from local over-injection causing large aliquots of fat [16,17]. Deprivation of the centrally located part of these aliquots from diffusion of nutrients and oxygen from surrounding tissues, subsequently, causes the process of "fat necrosis". And finally, the liquefaction of this necrotic tissue causes the clinical or radiological appearance of an oil cyst.

Radiological Safety

One of the main concerns that caused the 1987 ban [2] in regard to radiological safety was the fear that alterations on the different breast-imaging modalities might obscure or delay cancer (recurrence) diagnostics. Nowadays, it has been shown that distinctions can be easily made based on morphology, size and distribution provided that radiologists and surgeons communicate clearly. In **Chapter 2** we showed that despite the higher number of observations of fat necrosis following AFT when compared to standard oncoplastic breast reconstructions, an increased risk of missing a new or recurrent malignancy using standard radiological modalities (mammography, sonogram or MRI) could not be found. As was previously discussed, the oil cysts can be seen as result of fat necrosis and it is debatable whether this should be defined as a complication or a radiological finding. In addition to the process of fat necrosis, fibrosis, sclerosis and eventually calcification can occur in a period of 6 months- up to 10 years after treatment [18,19] and cause the characteristic appearances of micro- and macrocalcifications on mammography. Besides mammograms being the superior radiological modality for identifying calcifications, in **Chapter 3** we found sonogram's to be superior in identifying cysts and MRI in demonstrating fat necrosis. These findings were in line with other studies [13,20,21]. While radiological follow-up after AFT in breast surgery is not obscured in any way, biopsies to rule out the possibility of breast malignancy are still likely to be performed, either to relieve patient's anxiety or to avoid litigation [22]. It is a general believe amongst

experts that these difficulties will decrease over time with the ongoing advances made in radiological diagnostic accuracy.

AFT technique

The discussion on the AFT technique is extensive. However, often this is mainly a discussion on practical preference of the specific surgeon doing the procedure. Therefore, in this section of the discussion I would like to highlight the technical preferences of surgeons that perform AFT in high-volume centres and actively publish their results in the scientific literature. **Chapter 7** will give insights in all techniques used by a much larger number of European plastic surgeons, practising in smaller centres. For the sake of clarity AFT technique will be discussed for breast reconstruction and –augmentation combined.

The various important aspects of the AFT technique have been introduced in **Chapter 1** of this thesis and generally start with choosing your harvest location. While the abdomen and flanks are generally most often used because of easy accessibility and the avoidance of turning the patient peroperatively, there have been studies focussing on the preferable harvest location in terms of adipocyte viability. As was reported in **Chapter 2 and 3**, Saint-Cyr et al. [23], hypothesized on a better viability of the graft, when harvested from the trochanteric area, as a result of higher numbers of adipocytes and "colony forming units" (viable adipocytes in any given sample). However, a variety of other studies, both in-vitro as well as clinical [4,24-26], showed no relation between cell viability and harvest location. When discussing the infiltration of the harvest location a vast group of different solutions are currently either, on the market or prepared by the physician himself. Some studies have suggested a preference for anaesthesia solutions like lidocaine and ropivacaine over articaine or mentioned a preference for the absence of epinephrine [27]. However, a recent systematic review by Shim et al [28] showed no clear effect on adipocyte viability.

Besides the possible effect of infiltration solutions on the viability of the adipocytes and the stromal vascular fraction (SVF), the "(shear) stress" caused by the negative pressure of suction as well as the damage brought on by the laminar flow through the harvesting cannula are of great importance. Therefore, both the methods of harvesting as well as the harvesting cannulas are a subject of great scientific interest. The contradictions, regarding cannula-size, that currently exist between studies were briefly highlighted in **Chapter 2** by citing both Erdim et al. [29], and Ohara et al. [30] who described that 6 mm cannulas provide

both higher numbers of viable adipocytes as well as cause decreased graft survival (through the formation of larger fat lobules), respectively. Recently, Gonzalez et al. [31] showed that viable adipocyte cell count and both proliferation and enzyme-activity thereof, are not just cannula size depended but are also related to the number of side-holes the cannula has. Overall, there is still no substantial evidence to support the choice of one cannula size over the other. A number of studies have reported on the difference of adipocyte viability between manual (syringe) aspiration and various liposuction devices used for harvesting of the fat. He et al. [32]reported an 8.7% higher rate of adipocyte injury with a vacuum suction method compared to syringe suction but both Leong et al. [33] and Smith et al. [34], could not show a difference between the two harvesting methods. It should be noted that the cannula size varied greatly between these studies creating a reporting bias. However, Lalikos et al. [35] compared a small calibre cannula (2 mm) + syringe suction with a larger cannula (3 mm) + liposuction device and still found more viable adipocytes and less cell damage when using syringe suction. With this there seems to be a slight preference for manual aspiration using a syringe in terms of adipocyte viability and cell damage, but randomised trials comparing different suction methods and cannula sizes are clearly needed.

As was briefly highlighted in the introduction, preparation of the fat is an important step of the grafting process and besides centrifugation, as suggested by Coleman, can be achieved through washing (cotton, metal sleeve), decantation or filtration. Each of these methods aims at purifying the fat by disposing factors that can potentially compromise adipocyte viability, such as infiltration fluid, fibrous cords, unviable adipocytes, lipid droplets and blood [36]. Centrifugation, the most often reported form of preparation in **Chapter 2 and 3,** has been widely studied in both clinical as well as animal studies. Its potential has been advocated, amongst others, by Butterwick et al. [37], and Ferraro et al. [38]. Butterwick reported significantly longer survival and better aesthetic outcome compared to no centrifugation in hand rejuvenation and Ferraro compared two methods of centrifugation with decantation and found no fat absorption, after 12 months follow-up, in a significantly higher proportion of patients that received fat prepared through centrifugation at 1300 rpm for 5 min. This study showed a benefit of one particular centrifugation setting, a factor that is known to matter significantly as is shown in various studies. In regard to centrifugal forces – often reported as rotations per minute (rpm) – Kim et al. [39]., Xie et al. [40], and Kurita et al. [41], recommended not exceeding 3000-4000 rpm because of the risk of adipocyte damage and the absence of an additional purifying benefit.

The duration of centrifugation was studied by both Kim et al [39]., and Boschert et al. [42], in which the former warned of not exceeding beyond 5 minutes because of adipocyte damage and the latter advised not exceeding 2 minutes because longer sessions would not add in the cell viability. While seemingly a superior method of preparation, Rohrich et al. [25] found no difference in adipocyte viability with or without centrifugation and both Botti et al. [43], in their auto-controlled comparative study as well as in the animal studies of Ramon et al. [44], and Minn et al. [45], found no difference in adipocyte viability between centrifugation and gauze washing/ -filtration. On the contrary, both washing and decantation have also been proven superior methods of preparation over centrifugation. Khater et al. [46], reported a better clinical outcome after washing in a prospective controlled clinical study and both Conde-Green et al. [47], as well as Rose et al. [48], showed higher numbers of viable adipocytes after decantation besides less cell damage compared to centrifugation. Since no consensus on a superior method of preparation has been reached all methods are still being used in both experimental as well as clinical settings.

Recently, in an effort to further increase the adipocyte viability and its "take" in the recipient location, the use of supplementation has gained attention. While, for example, centrifugation itself can be seen as a method of enriching the lipoaspirate, the supplementation of fat with stromal vascular fraction or adipose stem cells has yielded larger fat grafts and longer survival [49-54]. The benefits - and possible dangers (see discussion **Chapter 6**) - of these forms of supplementation share a (cell) biological origin. The concept behind other forms of volume enhancing techniques like the BRAVA system are more mechanical. Using BRAVA and PALF which stands for "percutaneous aponeurotomy and lipofilling", the concept of "one-cupsize-augmentation-only" is now gradually subsiding, with breast augmentations up to the 250 ml range for A-cup patients now being attained [55,56].

Finally, the (re)injection technique. In general all clinical studies support the same transplantation approach which describes the technique of reinjection in a fanning, retrograde (on withdrawal), multilevel- and multi-tunnel manner. The theory behind this method stems from the understanding that fattish tissue – following its transplantation – lacks a native blood supply from the recipient bed and is therefore dependent of the osmotic effect of the surrounding host tissues for nutritive requirements. While physiological diffusion is limited to adjacent capillaries (150 μm) and revascularization requires at least 5 days, the adipocytes are in a stage of heightened metabolic demand and therefore very susceptible

to hypoxia. Maximum nutrition is therefore accomplished through this multi-level/ -multi-tunnel approach by allowing the fat granules of the smallest volume to have the largest surface of contact. This is thought to decrease the chance of accumulation of death adipocytes and thereby, fat necrosis [57]. A slow (0.5-1 ml/sec) and retrograde injection has been shown to add to this, in creating larger fat grafts as compared to higher injection speeds (3-5 ml/sec) [58]. One very important aspect in AFT in addition to both breast reconstruction as well as breast augmentation is the injection plane. As is further discussed in **Chapter 6** both the possible remaining dormant tumor cells after a radical resection as well as the capabilities of ADSC (related hormones) to promote tumor growth contribute to the current consensus that AFT following breast surgery should adhere to reinjecting into the subcutaneous and sub-fascial/ intramuscular planes only [59].

Efficacy: Volume retention/ Satisfaction

The mean volume retention – which is generally believed stable after 6 months [60] – was 76.8% and 62.4% for breast reconstruction **(Chapter 2)** and breast augmentation **(Chapter 3)** respectively. This resulted in a high patient- and surgeon satisfaction rates with the procedure, in line with the recent systematic review by De Decker et al. [61]. However, multicentre, patient specific questionnaires like the BREAST-Q for the quantitative measurement of patient satisfaction are lacking. Furthermore, multicentre, randomised clinical trials comparing different AFT techniques by measuring volume retention in a reproducible way, using a validated volumetric assessment tool like MRI, are clearly needed.

Finally, the author found that the following was worth discussing preoperatively prior to AFT for breast reconstruction but especially for breast augmentation. First of all the patient should be thoroughly informed about the residual swelling caused by postoperative oedema (50% after 4 weeks [15]) to avoid misconceptions about fat resorption, which stabilizes 4 to 8 months after treatment [60,62]. Secondly, the patient should be aware of the consequences of body weight fluctuations on fat graft retention [63] and thirdly, should be well informed about the fact that AFT is a volume enhancing technique that does not provide a breast-lifting effect for ptotic breasts and may actually accentuate ptosis [64].

Oncological safety of aft (in addition to breast surgery)

In **Chapter 6** the authors aimed to give a comprehensive overview of the scientific data currently available on this subject from both clinical- as well as basic science studies. However, due to the growing number of articles published on this subject and the significant depth of some of the basic science studies, certain elaborations are beyond the scope of this thesis, and I refer the reader to the respective relevant articles.

A large part of the basic science studies investigating the carcinogenic properties (i.e. tumour growth, migration, neo-vascularisation, self-renewal or metastasis) of AFT on breast tissue focus on co-culturing ADSCs and different breast cancer cell-lines using in-vivo or in-vitro techniques. Herein, three different direct pathways in which ADSCs can influence breast cancer cells, were described. First, gene receptors on the surface of breast cancer cells, encoded for by specific genes, can form a cross-link with ADSCs through specific growth factors which serve as an intermediary. Second, ADSCs can induce alterations on mesenchymal markers on breast cancer cells through epithelial–mesenchymal transition (EMT). EMT is a process by which epithelial cells lose their cell polarity and cell-cell adhesion, and gain migratory and invasive properties [65,66]. And third, ADSCs can secrete cytokines, chemokines and growth factors which can stimulate proliferation of breast cancer cells. In addition to the carcinogenic results from ADSCs in the direct vicinity of breast cancer cells, ADSCs were also reported to have migrating abilities towards breast cancer cells as well as vice versa. Finally, two more indirect manners by which AFT could promote cancer progression is by AFT instigated hypoxia and the immunomodulatory capabilities of ADSCs. However, the most interesting aspect about the immunomodulatory capabilities of ADSCs is that it also has tumerosuppresant abilities for one specific breast cancer cell line (MCF-7) through the expression of IFN-β.

During the discussion of **Chapter 2** we highlighted the most recent findings from clinical studies regarding oncological safety of AFT following breast cancer surgery. To summarize, in **Chapter 2** as well as in the recent systematic review by Waked et al [8]., the loco-regional and distant, oncological recurrence rate was considered low following both mastectomy and BCT patients. However, subgroup analysis of patients with certain confounding variables like hormonal breast cancer therapy or intra-epithelial forms of breast cancer still show higher rates of loco-regional recurrence following AFT [9-11]. Therefore, larger cohort, randomized clinical trials with sound methodology in analysing appropriate

subgroups according to these and other important parameters (radiotherapy, chemotherapy, lobular/ ductal, invasive/ in-situ carcinomas) are clearly needed. In the meantime, the noticeable discrepancies between these clinical studies and the before mentioned results from the basic science studies is another subject that qualifies for additional scientific clarification. As was discussed in **Chapter 6**, the main culprit herein is the sheer difference between the artificial biological environments created in the laboratory and the clinical setting. Banked breast cancer cell lines (BCCL's) in the laboratory, presumably undergone more mutations and are more durable than there clinical counterparts. Even more so, the concentration in which these BCCL's are cultured with lipoaspirate are presumably much higher since it is postulated that oncological recurrence following BCT can be the results of differentiation of a few remaining dormant breast cancer cells. The same applies the other way around because the concentration of both adipocytes and ADSCs, cultured with BCCL's, have been shown to be much higher than that of the average lipoaspirate [67-70]. So in order to narrow the gap between basic science- and clinical studies, one way to start is to use more clinically representable samples of both the different BCCL's as well as adipocyte and ADSCs concentrations. And finally, the second focus for further studies should be to more clearly define the "dormant" breast cancer cell and its characteristics in the vicinity of the lipoaspirate.

Aft in the treatment of scars

The application of AFT in the treatment of dermal scars started in the end of the previous century with Schuller et al. [71] and de Benito et al. [72] reporting on its use in 1997 and 1999 respectively. Back then it was mainly performed because of its volume enhancing properties in correcting contour defects and depressed scars. The histologic changes, including the improvement of skin quality, became apparent a decade later thanks to the work of Coleman et al. [73] amongst others [74-76]. Its properties in pain reduction have only recently been studied more thoroughly [77-79]. The interest in AFT in general is illustrated by figure 1 in **Chapter 5** and in **Chapter 4** we give an overview of the relevant studies for its use in the treatment of scar-related symptoms such as appearance and skin characteristics, volume depletion and contour irregularities, pain and itch.

One of the main features that burdens most patients, the scar appearance and its characteristics, improved significantly in terms of stiffness and thickness, col-

our, mobility, pliability relief, vascularization and pigmentation, after a 12 month follow-up. The volumetric restorative properties of AFT and its capability of improving contour deformities in a three-dimensional manner has also been thoroughly described but with great heterogeneity amongst studies and subsequently varying degrees (30-90%) of volume retention. In addition, the method of volumetric assessment varied greatly amongst studies. Therefore, larger multicentre trials comparing different methods of fat grafting, but with one validated volumetric assessment tool, like the Vectra3D Imaging System, are clearly needed. Regardless this lack of a validated, objective volumetric assessment, the patient's and surgeon's satisfaction with the technique, for both scar appearance and restoration of volume, was considered good. It is postulated that both are the result of stimulation of collagen fibre neo-synthesis [73-76]. Another debilitating feature of scar tissue is its lack of malleability, which – depending on the location of the scar – can cause functional impairment. Recent studies have suggested that the improvements in scar malleability following AFT might be the result of ADSCs, through stimulation of angiogenesis, local hydration and architectural remodelling [80,81]. Furthermore, for certain specific scars like after severe burns, degloving injuries or necrotizing fasciitis, the destruction of the subcutis and the subsequent need for autologous split skin grafts (SSGs) further enhances the chances of scar stiffness because of the absence of the "gliding" features of this subcutis [82,83]. In the recent study by Jaspers et al [84]., the authors showed a significant increase in scar elasticity, three months after a single AFT session, using two validated assessment tools (Cutometer and Patient and Observer Scar Assessment Scale [85-88]). They hypothesized that these results might be attributable to the presence of a new subcutis and follow-up studies might illustrate additional scar malleability-improving effects of AFT. Finally, the two more subjective properties of scars; pain and itch, were described in **Chapter 4** in seven and three studies respectively. Significant pain reduction with AFT was described mostly in relation to post-mastectomy pain syndrome besides general scars and after episiotomy, with a follow-up ranging from 6 to 13 months. Great heterogeneity was found between studies in the reduction of itching, with two studies reporting reduction and one study reporting no reduction at all. Therefore, larger randomized controlled trials are needed to support the claims that AFT derived mesenchymal cells can cause prolonged analgesia and thereby lead to a reduction in both scar related -pain and or -itch.

Aft in clinical practice

So far in this discussion we covered the scientific findings of a small group of innovators who publish their results, but clinically AFT is, of course, performed on a much larger scale. In order to narrow the gap between the findings from a few experienced surgeons and the many, relatively less experienced surgeons, we performed two survey studies to both ventilate the AFT experience, techniques used, and opinions of European surgeons and to compare the surgeon's satisfaction with that of patients.

Current European practice and opinion of AFT

With the steady increase in the number of scientific lectures about AFT during European plastic surgery meetings, the growing popularity of AFT in Europe is undeniable. Clinically, the technique is appealing to both patients and physicians because of the redistribution of fat and the relatively easy to perform technique, respectively. With the increasing number of larger cohort studies and the use of sound methodology, clinical guidelines on the use of AFT in general and specifically in breast surgery begin to emerge in various European countries [89,90]. The aim of these guidelines is to "guide" clinical practice, thereby making it safe and, to some extent, reproducible. One way to examine the degree and extent of its implication is through a survey, of which the results from 358 European plastic/ breast surgeons is presented in **Chapter 7**. Herein we found that overall the general experience of surgeons practicing AFT was relatively high, with more than a quarter of respondents having more than 20 years of clinical experience. Furthermore, the main application of AFT was in breast surgery and while most respondents performed only few procedures per year, the self-proclaimed experience was considered high. Considering the AFT technique, many respondents adhere to the methods described by Coleman [3,60,73] in accordance with previous survey studies [36,91]. However, deviations thereof, especially in the harvesting locations and techniques become more apparent and differ between countries. For example, the thigh is now reported a preferred (additional) harvesting location, especially in Belgium and France and a liposuction device instead of manual aspiration was preferred in 42% of cases overall. The first finding can hypothetically be the result from studies reporting on the quality of lipoaspirate harvested from specific regions such as the thigh [23]. The second - harvesting by use of a liposuction device - can have something to do with time-management but as was previously reported in this discussion, there

is a slight scientific preference for manual aspiration [28]. In addition, we previously reported on studies that described the importance of cannula sizes, both harvesting and reinjection. While no clear scientific consensus is achieved on this subject, it is something to reconsider as a surgeon performing AFT. Nonetheless, approximately 40% of the respondents who used manual aspiration for harvesting of the fat, indicated that they did not know the cannula size and this seems an area where further surgeon education might be appropriate. The most interesting findings, regarding AFT technique, discussed in **Chapter 7** were the deviations from AFT guidelines when it comes to injection planes. Herein, we found that intra-glandular injection of fat was still performed in implant based- and autologous flap reconstructions of the breast by 18.4 and 23.3% of respondents, respectively. Intra-glandular injection in the corrections of local breast defects after lumpectomy or partial mastectomy was even performed by 30% of the respondents and more often by more experienced surgeons. Considering the many indistinctness's regarding oncological safety previously discussed in this thesis, this seems to be the biggest, clinically unorthodox deviation from scientific recommendations. Henceforth, herein lies the greatest benefit from ongoing surgeon education.

Current surgeon-/ patient experience and –attitude

While many aspects of AFT are developing at an significant pace, the instruments we use to measure one of the most important aspects of its efficacy, namely satisfaction, is only recently beginning to evolve. Up until 2011 most studies only superficially mentioned good patient/ surgeon satisfaction with only a few using some sort of Likert Scale [20,92-97]. Since then, the value of patient reported outcomes measures (PROM) has gradually permeated in the world of AFT with several studies reporting patient satisfaction of AFT after breast reconstruction with either study specific PROM's [98] or validated questionnaires like the Breast-Q [99-101]. However, the Breast-Q, like other PROM's primarily reports on patient satisfaction. Therefore comparisons between the cosmetic evaluation of AFT from patients and surgeons cannot be made. At the same time a quantative objectification of the difference between what the doctor describes as "beautiful" and what the patient's perception is, might actually prove very helpful in the consultation room when discussing expectations preoperatively. In this respect in **Chapter 8**, we discuss the outcomes of our photo-comparison study. Herein the interrater agreement between 312 European plastic surgeons

and different patient groups are assessed based on the cosmetic evaluation of pre- and postoperative photographs of patients treated exclusively with BRAVA + AFT for various indications. The different groups of patients comprised of 43 patients after DIEP reconstruction, 20 patients following breast augmentation and 38 control group patients that never underwent any breast related procedures. Three sets of pre-/ postoperative photographs were scored based on the Harris Scale (HS; excellent, good, fair or poor) and included patients treated with BRAVA + AFT for three different indications. These indications were subsequently; (1) bilateral breast reconstruction after total prophylactic skin sparing mastectomy, (2) breast augmentation and (3) local defect correction after a lumpectomy. We found the highest correlation of cosmetic appreciation between the surgeons and the DIEP patients, followed by the control group patients. The lowest correlation was found between the surgeons and the augmentation group, which might therefore benefit from more extensive preoperative patient education. Furthermore, a high interrater agreement was found in the cosmetic evaluation of BRAVA + AFT amongst both surgeons from different countries as well as patients from different patient groups mutually. The authors were unable to find other photo-comparison studies let alone studies with a similar study design. Therefore, since no comparisons with the current literature could be made, further studies should focus more on the etiology of this intragroup consensus (patients and surgeons mutually) and intergroup disparity (patients vs surgeons) in order to improve doctor-patient communications. Finally, when looking at the increase in appreciation of the postoperative photographs relative to the preoperative photograph, i.e. the scoring trend, we found that patients tend to be more optimistic regarding the results than surgeons. This is probably the result from differences in appreciation, with patients judging the appearance from an emotional standpoint and surgeons much more from a technical point of view. But, with no comparable studies, we believe that further studies should focus much more on the qualitative characteristics of these differences. However, to achieve this goal, special, more specific (qualitative), surgeon reported outcome measures (SROM's) have to be developed that allow direct comparison with PROM's.

Aft as a viable tool in facial rejuvenation

Finally, I would like to conclude the discussion of this thesis with another upcoming indication of AFT; namely its application in cosmetic facial rejuvenation.

Over centuries, the face has always been considered one of the most prominent features of a human being; the way we identify ourselves to the world. Therefore, it is not surprising that one of the founders of modern AFT, dr Coleman, first described the application of AFT in the treatment of the periorbital region [3]. However, the real upsurge of scientific papers written on the subject stems from quite recently, parallel with the steady decline of purely surgical procedures to the face (i.e. face-/ MACS lift) [102,103]. Nowadays, many authors publish either personal results, with varying sample sizes, from their preferred method of facial rejuvenation using AFT or a descriptive review of the literature [104-118]. It is, however, the author's experience that a systematic review with meta-analysis, reporting on the rejuvenating properties of AFT to the face in terms of volume enhancement, complications and patient/surgeon satisfaction, is currently lacking. Therefore, the results from such a study, performed by the authors, is reported in **Chapter 9.** Herein, we found that the AFT techniques used varied greatly, between authors, similar to the use of AFT for other indications. Amongst these variations in AFT techniques were infiltration solutions, harvesting methods, cannulas and methods of preparation, all essential for the final result for reasons previously reported in this discussion. Dermal fillers in general have known a variety of both minor and major complications and one of the possible benefits of AFT over other dermal fillers is the absence of certain specific complications like hypersensitivity reactions or granuloma formation. Presumably because of the biocompatible nature of lipoaspirate, these complications were, indeed not reported nor where other major complications like skin necrosis or blindness due to thrombo-embolic events. However, thrombo-embolic events are of course much more operator dependent, especially near the so-called "danger zones" of the nose [119] and larger studies are needed to confirm this. Overall, the complication rate was considered low (6%), with hematoma/ ecchymosis occurring in 5% of cases and the only AFT specific complication (fat necrosis/ oil cyst formation) in 2% of cases. However, data regarding volume retention could not be pooled because of heterogeneity between studies. This expected heterogeneity, stems for a large part from the fact that these studies described different grafted facial zones which all differ significantly in their density and histological make-up. Further studies should therefore focus on facial location-specific volumetric assessment using objectifiable tools like 3D imaging (such as the VECTRA XT® 3D imaging system), CT or MRI. Even so, meta-analysis of the conjoined satisfaction rates showed a 81% patient satisfaction with facial AFT, next to a 89% surgeon-reported good cosmetic result. In

conclusion, AFT seems to have real potential as an alternative biocompatible facial filler but proper research with long term follow-up regarding facial-zone-specific-volume-retention is needed to prove its superiority over the conventional fillers.

The common thread

As is revealed in this thesis AFT is an innovative technique of which I, the author, feels it has the potential to become one of the major new inventions in reconstructive surgery. The expansion of its use to other indications and surgical fields during the last decade as well as during the course of my PhD has attributed to the diversity of articles included in this thesis. It is, however, interesting to conclude this discussion with a recapitulation of what makes AFT such an innovative technique. In other words "*the common thread".*

What defines AFT is that from which it originated as well as that which forms the common ground in all different indications, namely; the desire to correct an absence of organic volume through autologous replacement. It accomplishes this through an ever evolving series of steps in which native fattish tissue is harvested, processed and reinjected. The absence that AFT aims to correct can be congenital (Poland Syndrome), iatrogenic (depressed surgical scars, post-mastectomy), traumatic (burn victims, deglovement injuries) or acquired (loosening/ sagging facial skin). One of the most important clinical and scientific questions regarding AFT is its safety. However, the definition of "safety" differs per indication. Due to the ADSCs and the hormonal factors that are transplanted along with the adipocytes, safety concerns in breast surgery is mainly directed at possible cancerous differentiations in glandular tissue. While these concerns are of a much more cell-biological nature, the safety of AFT in facial/ hand rejuvenation or in the treatment of Dupuytren's disease [120] is much more mechanically orientated. Herein, the consecutive insertions of the cannula in compact spaces, rich in various crucial anatomical structures, is far more important and safety herein is therefore much more operator dependent. In the scientific literature, the efficacy of AFT is mainly reported in terms of satisfaction and by quantifying the amount of volumetric correction/ augmentation that remains after a certain amount of time, e.g. volume retention. The appreciation of both patients and surgeons as well as the volume retention seems satisfactory. However, the majority of studies reporting on the former lack validated questionnaires and the

latter is plagued by poorly reproducible methods of volumetric assessment. Finally, a special feature of AFT, one that is being increasingly reported on, is the improvement in scar and skin trophicity. This process is presumably due to the stimulation of collagen neo-synthesis and angiogenesis caused by AFT and with this the technique transcends above just being a space occupying modality. So to conclude, AFT is rapidly evolving to become an important asset in the surgeons repertoire of reconstructive modalities. Consequently, with time and answers on important questions like safety and efficacy it may turn out that AFT could be rightfully called the liquid gold.

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Chapter 11

Valorization Addendum
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Valorization addendum

Introduction

Knowledge gathered through scientific research is particularly of value when it serves some form of socio-economic purpose. The description of value, or "valorization", is imperative in justifying the costs that are involved with scientific research, costs that are often financed by public authorities, government bodies or through funding. In this chapter the valorization of this thesis and the PhD-trajectory that preceded it will be discussed in the light of its relevance, target audiences, products or services, innovation and realization.

The primary focus of this thesis has been on Autologous Fat Transfer (AFT) in breast surgery, which – for the purpose of this discussion - can be divided in breast reconstruction and breast augmentation. The main reason for breast reconstruction is breast cancer, which is still the most common form of cancer in women worldwide with over nearly 1.7 million new cases diagnosed in 2012 [1]. In the Netherlands this translates to one in eight women, but fortunately early screening and targeted therapies have greatly improved survival [1,2]. Numbers on (type of) breast reconstruction performed in the Netherlands are scarce and vary greatly but overall it is thought that 30-50% of Dutch women undergo mastectomy and that less than 15% thereof undergo breast reconstruction. The annual rate of some form of breast conservative therapy (BCT) is currently estimated at 60% [3]. The primary goal of both forms of reconstruction (following mastectomy or BCT) is obtaining the best cosmetic results since unfavorable cosmetic outcomes have been proven to significantly decrease the quality of life and psychosocial functioning [4,5]. Breast reconstructive options generally encompass the use of implants or autologous tissue. The latter can exist of various free or pedicled flaps following mastectomy or volume displacement and replacement techniques following BCT. However, using autologous tissue in breast reconstruction is not flawless. In some studies complications rates following DIEP reconstruction and poor cosmetic outcome following dis-/replacement techniques both reach 30% respectively [6-10]. Furthermore, the widespread use of silicone breast implants in both reconstruction and augmentation has recently, again, been questioned due to new discoveries in both ASIA syndrome [11] as well as BIA-ALCL [12]. These doubts about silicone implants, brings us to the second focus of

this thesis in regard to breast surgery, namely; breast augmentation for cosmetic benefit. Despite the forewarnings about ASIA syndrome and BIA-ALCL the number of women who underwent breast augmentation in the Netherlands is actually increasing. According to the Dutch Breast Implant Register (DBIR), the number of breast operations with the use of implants increased from 6000 in 2013 to 9000 in 2015 [13]. Furthermore, out of the 13.600 patients (25.500 implants), that have been recorded between April 2015 and December 2016, 75% was treated for cosmetic purposes [13].

In conclusion, in plastic surgery breast reconstruction and breast augmentation are the most frequently performed reconstructive and cosmetic procedures respectively. AFT is a potential new technique which may complement or (completely) replace existing techniques of breast reconstruction/ augmentation. Therefore, AFT could potentially become an important attribute to the armamentarium of the breast surgeon.

Relevance of scientific results

The relevance of this thesis on breast surgery lies mainly in the endorsement of AFT as a novel technique. A technique that has fewer complications and the potential to not only complement current methods of breast reconstruction and augmentation but also to – one day – replace them. We were able to highlight both the efficacy in terms of volume retention and patient's/ surgeon's satisfaction and the safety by illustrating currently acceptable oncological recurrence rates. Furthermore, by elaborating on hiatuses between basic science- and clinical studies as well as between the performance of AFT pioneers and less experienced surgeons, we provided a base for future studies. The results from these future studies bring us one step closer to successfully implementing this technique in mainstream breast surgery. It is currently too soon to make comparisons in cost-effect analysis between AFT and other forms of breast reconstruction/ -augmentation. However, a recent study by Sorin et al. estimated the costs of a single 500ml AFT procedure, with their apparatus, at 9.28 euro, or 10.52 USD. Thereby, this thesis is considered to be relevant for future female patients seeking breast reconstruction or augmentation, as well as in reducing socio-economic costs.

Target population

The results of this thesis are relevant for researchers and other professionals in the medical field with special interest in breast reconstruction and augmentation. In addition, this thesis is of interest to breast surgeons, surgical oncologists, plastic surgeons and dedicated nurses.

Activities and products

The beauty of a thesis regarding the use of AFT is that it does not directly translate into a certain activity or more importantly; a product. The "product" that is used in AFT is – as the name suggests – "autologous", meaning it is merely redistributed, without the addition of synthetic material. However, this does not limit its potential since the AFT "activities" – or better indications – are numerous, ranging from volumetric enhancement to the correction of surgical defects.

Innovation and realization

The use of AFT in addition to or instead of conventional breast reconstruction or augmentation is innovative on its own, i.e. it preludes a whole new chapter in the development of breast surgery. As was stated in this thesis there are still remaining questions to be answered such as on oncological safety and follow-up on volume retention. Nonetheless, through the various benefits of AFT that have been highlighted in this thesis, the authors, emphasized the undeniable place AFT will take in the future repertoire of breast reconstructive possibilities. As was discussed in the introduction current breast reconstructive options to restore lost volume, consist mainly of the use of either local or distant autologous tissue or breast implants. Herein, AFT offers numerous options to both aid or totally replace these forms of reconstruction. The former is already practiced on a large scale with final "touch-up" AFT corrections of small local defects being common practice in certain centers. The latter will certainly gain more recognition when volumetric results from mega-volume enhancement techniques like BRAVA [14,15] become more apparent. Therefore, the author believes that with the results from this thesis a more prominent role will be reserved for AFT in future breast reconstruction, clinical guidelines.

The endorsement of AFT in the Netherlands is an ongoing process that has already known more than a few hiccups along the way in terms of insurance coverage. Currently, it is mainly hospital funded and on a scientific bases, such as in the case of the BREAST-trial (ClinicalTrials.gov identification number NCT02339779) [16]. Furthermore, it is self-funded by patients in various clinics nationwide for cosmetic purposes but with ongoing developments we hope to demonstrate its added value and achieve full insurance coverage and implement this promising technique in reconstruction patients also.

Facial rejuvenation and scars

Besides the indications for AFT in breast surgery, I want to briefly highlight the value of its use in facial rejuvenation and in the treatment of scars. Currently, facial rejuvenation is realized with surgery and increasingly with the use of dermal fillers. While the use of dermal fillers can be seen as a strictly cosmetic procedure, they can also sometimes be utilized in reconstructive procedures of the face. None of the currently available fillers are beatific and since its use is expanding it is appropriate to explore the opportunities of AFT as a potential, long-term, biocompatible filler.

In regard to scars, it has been illustrated in this thesis, that scars can have debilitating effects on a person's quality of life and that important aspects of a scar were positively influenced by AFT. Therefore, this thesis provided a stepping stone in the process of further investigations on the scar-/ skin healing properties of AFT.

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Summary

Chapter 1 is a general introduction to autologous fat transplantation (AFT) and the topics in this thesis. This chapter forms the background against which the aims and research questions are explained

Chapter 2 is a meta-analysis, that gives an overview of the efficacy and safety of the use of AFT in onco-plastic breast reconstruction. In this chapter the results from 43 studies are reported in terms of indication, volume retention and patient-/ surgeon satisfaction. Furthermore, safety of the procedure is reported in terms of the oncological- and radiological safety besides the complication rate. Loco-regional and distant oncological recurrence in patients who underwent breast reconstruction in conjunction with AFT were 2.5 and 2.0% respectively, which is considered lower than that following similar procedures without AFT. The total complication rate of 8.4% is also considered lower than other forms of breast reconstruction and radiological findings after AFT are easily distinguished from other pathology. There was however a higher rate of biopsies based on radiological findings following AFT all of which were benign. Patient- and surgeon satisfaction was considered high and the mean volume retention of 76.8% seems promising.

Chapter 3 is a succeeding systematic review that further elaborates on the efficacy and safety of AFT in conjunction with breast augmentation for cosmetic purposes. Twenty-two articles, covering a total of 3565 patients with a follow-up of 12 to 136 months reported a total complication rate of 17.2%. Complications most frequently comprised of indurations, persistent pain or hematomas and mammograms revealed micro- or macrocalcifications in 9% and 7% respectively. Calcifications were however, still easily distinguished from those that can be found in breast cancer and the mean volume retention was 62.4%. Patient- and surgeon satisfaction was considered high.

Chapter 4 gives an overview of the applications of AFT in the treatment of scars. In a systematic review of 26 articles a significant improvement of scar appearance, skin characteristics and pain was reported. Subsequently, an improving trend of itch, volume restoration and three-dimensional contour was found. However, no meta-analysis could be performed due to heterogeneity between studies in methodology as well as reporting outcomes.

Chapter 5 contains a reply to a letter to the editor, further elaborating on the positive effects of AFT on scar tissue and scar-related conditions. Herein, we further discussed the extend of the AFT possibilities for different types of scar tissue (i.e. Morbus Dupuytren) as well as hypotheses regarding the mechanisms through which the positive effects of AFT are orchestrated. Finally, we discussed the difficulties with reimbursement from insurance companies and an upcoming Dutch, multicenter, RCT comparing external preexpansion and AFT versus the conventional tissue-expander/ implant-based breast reconstruction in mastectomy patients.

In **Chapter 6** the authors elaborated on the subject of oncological safety by comparing results from clinical studies with the outcomes of 35 basic science studies. What stood out were the significant discrepancies between results from clinical studies declaring the relative safety of AFT and the results from the basic science studies. The latter comprised mainly of studies that co-cultured different breast cancer cell lines in-vitro or in-vivo (mouse models) with ADSCs from lipoaspirate and reported on the different pathways through which tumor growth, -progression or metastasis were orchestrated. One of the main culprits in the comparison of the results from clinical and basic science studies originate in the significant differences in cell-biological environment between cultured laboratory- and clinical "real life" settings. Therefore, both specific subgroup analysis in clinical studies as well as better real-life simulating laboratory settings in basic science studies are needed to make conclusive statements about the oncological safety of AFT.

Chapter 7 investigates the AFT experience, -techniques used and -opinions of 358 plastic/ breast surgeons from 10 European countries. In this survey study it was shown that breast surgery is still the most prominent indication for which AFT is used by plastic surgeons, and that most adhere to the Coleman Method. However, deviations thereof are becoming more apparent, with for example the liposuction device instead of manual aspiration for harvesting of the fat, used in 41.9% of overall respondents. Furthermore, it was shown that intraglandular AFT is still performed in both implant- as well as flap based breast reconstruction besides local defect corrections, such as after lumpectomy. Given the uncertainties that still exists regarding the oncological safety of AFT and the fact that current clinical guidelines advice against intraglandular fat injection the authors argued that further surgeon education on this subject might be beneficial.

Chapter 8 further elaborates on the experience from surgeons and patients with AFT by comparing the cosmetic appreciation of different groups from both. European surgeons and Dutch patients following both DIEP as well as augmentation procedures of the breast (besides a control group), were asked to evaluate pre-/ postoperative photographs of BRAVA + AFT for various indications. The interrater agreements that were found between groups varied from high (between surgeons and patient groups mutually) to very low (surgeons vs augmentation patients). In order to further improve doctor-patient communications future studies should focus more on the etiology of these different consensuses and disparities, with emphasis on the qualitative characteristics thereof.

Chapter 9, concludes the thesis with the investigation of another upcoming indication of AFT, namely its use in facial rejuvenation. Through a systematic review with meta-analysis of 18 articles, covering 3073 patients the authors found that the procedure is generally met with few complications and high patients satisfaction rates. Furthermore, certain filler specific complications like hypersensitivity reactions and granuloma formation are unlikely, due to the biocompatible nature of AFT. However, larger cohort studies are needed before the same can be said about more operator dependent major complications like skin necrosis and thrombo-embolic events.

Chapter 10, entails the general discussion, in which the results that were presented in this thesis are discussed in the light of the current scientific literature.

Nederlandse samenvatting

Hoofdstuk 1 bevat de algemene introductie over autologe vet transplantatie (AFT) in relatie tot de hoofdstukken uit dit proefschrift. Dit hoofdstuk beschrijft de achtergrond waartegen de doelen van dit proefschrift alsmede de onderzoeksvragen worden uitgelegd.

Hoofdstuk 2 bevat een meta-analyse. Hierin wordt een overzicht gegeven van de efficiëntie en veiligheid van AFT als procedure in borst reconstructies. In dit hoofdstuk worden de resultaten uit 43 studies besproken in termen van indicatie (reden van AFT behandeling), volume retentie (achterblijvende deel ingespoten vet) en patiënt-/ chirurg tevredenheid. Daarnaast bespreken we – in het kader van veiligheid – de complicaties naast de oncologische veiligheid en de radiologische veiligheid. Het principe van de oncologische veiligheid omvat alle vragen met betrekking tot of- en hoe de toepassing van AFT in de reconstructie van een borst (na de behandeling van borstkanker) opnieuw voor het ontstaan van kanker kan zorgen. De radiologische veiligheid van AFT gaat over de mate waarin veranderingen in borstweefsel, door toedoen van AFT, het beeld van een dergelijke borst op echo-, mammogram of MRI dusdanig verstoren dat een radioloog deze borst niet meer goed kan beoordelen. Lokale terugkeer van borstkanker en terugkeer van borstkanker op afstand (bijvoorbeeld een metastase) werd gezien in respectievelijk 2.5 en 2.0% van de patiënten die AFT ondergingen in het kader van een borstreconstructie. Deze aantallen waren lager dan de aantallen die beschreven worden in de literatuur over patiënten die (verschillende vormen van) borstreconstructies ondergingen, zonder de toevoeging van AFT. Het totale percentage complicaties van 8.4% was tevens lager dan dat na andere vormen van borstreconstructies. De radiologische veranderingen op mammogram, echogram of MRI bleken makkelijk te kunnen worden onderscheiden van andere ziektebeelden, waaronder borstkanker. Echter, door toedoen van onzekerheid over radiologische bevindingen blijkt er na AFT nog wel vaker sprake te zijn van biopsies. Deze biopten bleken achteraf allemaal echter wel benigne (goedaardig). De tevredenheid onder chirurgen en patiënten was hoog en de gemiddelde volume retentie betrof 76.8%.

Hoofdstuk 3 is een voortzetting van hoofdstuk 2. Hierin worden opnieuw de resultaten vanuit een systematische review uiteengezet in termen van efficiëntie en veiligheid, zij het ditmaal over het gebruik van AFT bij een borstvergroting

voor cosmetische doeleinden. Uit 22 artikelen, die rapporteerden over een totaal van 3565 patiënten, met een follow-up van 12-136 maanden, bleek een complicatie voor te komen in 17.2% van de gevallen. Vaak betrof het een induratie (verharding), pijn of een hematoom. Mammografieën toonden micro-/ macrocalcificaties in 9% en 7% van de gevallen respectievelijk maar konden makkelijk gedifferentieerd worden van calcificaties passende bij borstkanker. De gemiddelde volumeretentie in deze studie betrof 62.4% en de patiënt-/ chirurg tevredenheid was wederom hoog.

Hoofdstuk 4 bevat een systematische review waarin de behandeling van AFT tegen verschillende klachten behorend bij littekens uiteen wordt gezet. Uit 26 artikelen bleek een significante verbetering in de vormgeving van het litteken, alsmede karakteristieken van de omliggende huid en een vermindering van pijn. Jeuk, volume en contour lieten ook een verbeterende trend zien, maar meta-analyse kon niet worden verricht vanwege grote heterogeniteit (verschillen) tussen de studies.

Hoofdstuk 5 bevat een toelichting op hoofdstuk 4, als reactie op een "letter to the editor". Hierin worden kort uiteengezet; de overige indicaties voor AFT (o.a. Morbus Dupuytren) alsmede de huidige stand van zaken met betrekking tot vergoeding voor AFT door de zorgverzekeraar.

In **hoofdstuk 6** wordt dieper ingegaan op het vraagstuk; oncologische veiligheid. Hierin wordt een vergelijking gemaakt tussen klinische studies en laboratorium studies. Zoals blijkt uit hoofdstuk 2 blijkt de kans op terugkeer van borstkanker door toedoen van AFT, uit klinische studies, klein. Echter, uit laboratorium studies waarin verschillende soorten borstkanker cellen gekweekt worden met stamcellen en hormonen - afkomstig uit vetweefsel – blijkt een snellere groei en metastasering van deze borstkanker cellen. Aan de andere kant blijkt uit een aantal andere studies dat er soms ook sprake kan zijn van verminderde groei van borstkanker cellen, juist door toedoen van factoren afkomstig uit het ingespoten vetweefsel. Concluderend kunnen we zeggen dat er momenteel veel nog niet bekend is over de mogelijke kanker stimulerende of –remmende werking van AFT. Wat we wel weten is dat er veel verschil bestaat tussen het celbiologische milieu in de laboratoria setting en de patiënt. Het vinden van antwoorden betreffende oncologische veiligheid ligt daarom enerzijds in het analyseren van subgroepen patiënten met verschillende soorten borstkanker, behandeling en hormonale status. Anderzijds dient er gestreefd te worden naar een beter

klinisch representatieve laboratoria setting, met betrekking tot aantal en type gekweekte cellen alsmede het milieu waarin.

Hoofdstuk 7 gaat verder in op enkele belangrijke aspecten van AFT die reeds eerder in hoofdstuk 2 en 3 aan de orde zijn gekomen. In dit hoofdstuk worden zaken als AFT techniek en de mening van chirurgen echter belicht vanuit de dagelijkse praktijk. Met behulp van een online vragenlijst, onder 358 chirurgen uit 10 Europese landen werd duidelijk dat AFT nog steeds het meest toegepast wordt bij operaties aan de vrouwelijke borst. De Coleman methode is nog steeds de meest gebruikelijke techniek maar hier wordt steeds meer van afgeweken, bijvoorbeeld door het gebruik van liposuctie machines (41.9%) in plaats van het handmatig oogsten van vet met behulp van een injectiespuit. De meest opvallende bevinding betrof wel het aanhoudende gebruik van intra-glandulaire AFT, of te wel het injecteren in het borstklierweefsel. Dit bleek nog steeds te worden toegepast bij zowel autologe flap reconstructies als reconstructies met borstimplantaten en in correcties van lokale defecten van de borst. Dit is opmerkelijk gezien de onzekerheden met betrekking tot oncologische veiligheid van AFT en het feit dat mede hierom de klinische richtlijnen intra-glandulaire AFT afraden.

In **hoofdstuk 8** wordt het onderwerp patiënt en chirurg tevredenheid nader belicht. Hierin wordt de cosmetische waardering die beide groepen geven aan verschillende indicaties van AFT met elkaar vergeleken in het verkrijgen van een "(interrater) agreement". Van Europese chirurgen en verschillende patiënten groepen (postoperatief na DIEP reconstructie, na borst augmentatie en een controle groep) werd gevraagd foto's van voor en na een operatie met BRAVA (breast enhancement and shaping system) + AFT te beoordelen middels de Harris Schaal (Matig, Redelijk, Goed, Uitstekend). De "interrater agreements" die hiermee gevonden werden varieert van hoog (tussen chirurgen onderling en patiënten onderling) tot zeer laag (tussen chirurgen en patiënten na borst augmentatie). Om de communicatie tussen de arts en de patiënt verder te verbeteren moeten toekomstige studies zich richtten op de oorzaken en kwalitatieve karakteristieken van deze verschillen tussen artsen en patiënten.

AFT wordt in opkomende mate toegepast als "filler" voor het verkrijgen van een jonger, levendiger gezicht en in **hoofdstuk 9** worden belangrijke uitkomsten hiervan nader toegelicht. Uit een systematische review met meta-analyse van 18 studies en een totaal van 3073 patiënten bleek deze procedure gepaard te gaan met weinig complicaties en hoge patiënt tevredenheid. Een bijkomend voordeel

van AFT is de afwezigheid van complicaties die specifiek zijn voor synthetische "fillers" zoals granulomen en overgevoeligheidsreacties. Echter, meer zeldzame, ernstige complicaties zoals huidversterf of blindheid door toedoen van tromboembolische processen (afsluiten bloedvat door stolsel) zijn meer chirurg afhankelijk. Deze complicaties zijn – hoewel nog niet beschreven na AFT – nog onvoldoende onderzocht.

Hoofdstuk 10 bevat de discussie van dit proefschrift. Hierin worden de resultaten nader besproken en afgezet tegen de meest recente wetenschappelijke literatuur.

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List of publications

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About the author

Jan-Willem Groen was born on September 15th, 1984 in Hoogeveen, The Netherlands. After finishing the Lower General Secondary Education (MAVO) in 2000, he started nursing school at the Alfa College, Hoogeveen in aspiring to become a paramedic. Upon finishing in 2004 - during which time he was heavily involved in competitive sports - came the ambition for a more challenging occupation which was found in the Dutch Royal Army. Early 2015, after passing the strenuous physical and mental testing, he was admitted to the Korps Commando Troepen (Dutch Special Forces) training program amongst 80 out of more than 200 applicants. Due to an ongoing calf injury he was unable to continue after 16 weeks and was honorably discharged on medical grounds. During his own physical rehabilitation he became interested in physical therapy of which he started the educational training program at the Higher Vocational Education (Hanzehogeschool), Groningen. During this time he became increasingly interested in (para)medical topics beyond the scope of musculoskeletal disorders only. It was during this time that he became to aspire a medical career which was now – for the first time – a viable option in the form of the Premaster Medicine at the Rijks University Groningen. This program provides a chance for aspiring physicians, who completed another higher vocational or academic form of education, to complete the Dutch Medicine training program in 4 years. In order to comply with the admission requirements the author – during the last two years of training in physical therapy – acquired the high school certificates for physics and chemistry before enrolling in the premaster entrance examination. Admission is based on highest score on the entrance exam and after graduating with a ninth place of a total of 380 applicants the author started his medical education in September 2009.

After finishing the first year, which functions as an transition program, the author was admitted to the master program and started his clinical internships. During this period he published his first scientific article for the department of Orthopedics titled: *"After total knee arthroplasty, many people are not active enough to maintain their health and fitness: an observational study"*. Upon finishing his final clinical rotations at the department of general- and cardiothoracic surgery respectively at the "Onze Lieve Vrouwe Clinics", Amsterdam he started

his residency at the department of cardiothoracic surgery at the Erasmus MC, Rotterdam.

In collaboration with the Vrije University Medical Center but simultaneous with his clinical work the author continued his scientific efforts with two systematic reviews about the efficacy and safety of AFG in addition to breast-reconstruction and –augmentation. After 2,5 years of cardiothoracic residency at the Erasmus Medical Center, Rotterdam and the Saint Antonius Medical Center, Nieuwegein respectively Jan-Willem made the transfer to the plastic surgery department of VieCuri Medical Center, Venlo and Zuyderland Medical Center, Sittard subsequently. It was during this period that the additional projects in what would later become this thesis, transpired.

After one year of plastic surgery residency and a number of both national as well as international recitals regarding the subject of this thesis the author received a €7400,- Grant for promoting scientific partnership between VieCuri Medical Center and Maastricht University Medical Center.

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