

# Autologous Fat Grafting – Factors of Influence on the Therapeutic Results

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## 1. Introduction

The correction of soft tissue defects has always been a challenge for plastic surgery. In the different stages of its historical development these problems has been solved by a variety of complicated techniques or filler materials some of which proved in time not only to be ineffective but even harmful for the human body. The ideal filler should be physically and chemically stable, long lasting and immobile in body, nonimmunogenic, noninfectious, nonpyogenic and nonallergic, should not require pretesting and should be cheap and easily stored. Some of these characteristics has been defined back in 1953 (Scales, 1953) but still there is no consensus regarding the perfect injectable material. Nowadays, despite of the growing number of alloproducts, the autologous fat grafting is recognized as one of the basic methods for soft tissue defects correction. It represents a three staged procedure of aspiration, purification and reimplantation of fat cells (Mojallal et al, 2004). The considerable presence of adipose tissue in the body and its autogenous origin guarantying advantages as biocompatibility, structure stability and polyvalent usage are reasons for the growing popularity of this intervention and its broadening spectrum of indications. Nevertheless there are still a lot of controversies and unsolved questions regarding the autologous fat grafting. Its efficiency is still often doubted due to the variable resorption rates and the unpredictability of the post operative outcomes. In attempts of optimizing the results many surgical techniques have been created (Billings at al., 1989; Mojallal, Foyatier, 2004). The new concepts were built on attempts to avoid excessive pressure changes during harvesting and reimplantation, improving the means of purification from potential local inflammation promoters, and application in a fashion that assures sufficient nutritional sources (Mojallal, Foyatier, 2004; Shiffman, Mirrafati, 2001). Though none of the newly invented techniques is considered optimal a consensus has been reached on some stages of the technical implementation of autologous fat grafting such as manual syringe lipoaspiration and three-dimensional reimplantation (Niechajev, Sevcuk, 1994; Coleman, 2001; Jauffret et al., 1994; Lalikos et al., 1997; Moore et al., 1995; Novaes et al., 1995). To date the optimal method of transplant purification still remains unclear as well as the impact on the therapeutic results of factors as combination with other interventions, intraoperative extent of correction and the need of numerous transplantations as methods counteracting the postoperative resorption. At the same time the growing knowledge on adipose tissue and the revealed

importance of the preadipocytes for transplant survival set questions about the influence of factors as age and gender, indications (esthetic or reconstructive), recipient site, etc. These and other issues will be discussed in this chapter as the profound knowledge on the existing techniques, the characteristics of the transplant and the influence of certain factors on therapeutic results could contribute to one's outcomes or technique refinement.

## 2. Surgical characteristics of adipose tissue

Adipose tissue is a particular sort of connective tissue existing in three different in morphology and function types. These are: white, brown and medullar adipose tissue which under the influence of various stimuli could turn into one another (Ashjilian et al., 2002; Casteilla et al., 2004; Mojallal, 2003; Jauffret, 1998).

The white adipose tissue is of greatest surgical importance. In people with normal body mass index it constitutes 15%-20% of the mass in males and 25%-30% in females (Ryan et al., 1989). It is scattered or distributed in depots localized in various anatomical regions according to age, gender and different nutritional and hormonal stimuli.

Morphologically white adipose tissue consists of stromovascular fraction and lipid inclusions containing mature adipose cells called adipocytes. Adipocytes together with hondrocytes and miocytes originate and differentiate from a multipotent cell of a mesodermal type (Ashjilian et al., 2002; Mojallal, 2003; Jauffret, 1998). The mature adipose cells have a roundish form and a diameter varying between 10 $\mu$ m and 120  $\mu$ m. They are known as unilocular fat cells as their lipid inclusions are organized in one big lipid drop occupying almost the entire cell and pushing the nucleus and cytoplasm to the periphery. In the adipose tissue they are structured in lobules divided by connective tissue fibers.

The stromovascular compartment of adipose tissue contains nerves, capillary vessels, connective tissue fibers, fibroblasts, macrophages and endothelial cells localized in the interadipocytary spaces and immature multilocular fat cells called preadipocytes. Single preadipocytes could be found in the fat lobules among adipocytes as well. The immature adipose cells strongly resemble the fibroblasts' morphology. In comparison with adipocytes they are smaller with centrally placed nucleus and a great number of little lipid droplets. Unlike the mature adipose cells, preadipocytes have the ability to divide and proliferate into adipocytes and thus are realizing the process of adipogenesis, now recognized to extend throughout life (Atanassova, 2000; Dugail, Ferre, 2002; Casteilla et al., 2004)

Nowadays, the growing knowledge on the complexity of adipose structure and functions revealed great potentials of influence on surgical results. Though fat tissue was initially accepted as passive energy storage, it is now clear that it is a major endocrine and paracrine organ producing a variety of hormones and signalling molecules, generally referred to as adipokines (Mohamed-Ali et al., 1998; Fruhbeck et al., 2001; Chaldakov et al., 2006; Tore et al., 2007). Leptin is recognized as a hormone of great importance for the process of auto regulation, assimilation of nutritional sources, puberty and fertility (Zhang et al., 1994, Montague et al., 1997; Lafontan, Bouloumie, 1999). It is synthesized mainly in adipocytes and in very small quantities in preadipocytes. This makes it an original marker for identification of mature and immature fat cells (Markman, 1989; Atanassova, 1996; Lafontan, Bouloumie, 1999; Atanassova, Popova, 2000; Ashjilian et al., 2002). Of greatest importance for the autologous fat transplantation is the participation of leptin in the process of

angiogenesis. The hormone exerts a strong stimulating effect on endothelial cells and consequently on graft microvascularization and fat tissue de novo synthesis.

The considerable presence of adipose tissue in the human body is a great advantage for autologous fat grafting. Its distribution in the anatomical regions is very specific and it strongly influences the choice of donor sites (Ryan, Curri, 1989; Jauffret, 1998). Adipocytes' number and size vary according to their localization due to the differences in blood supply and the response to neuroendocrine factors (Hudson et al., 1990; Dugail, Ferre, 2002; Casteilla et al., 2004). Y. J. Illouz describes two types of adipose tissue: an "ordinary" – which chemical homeostasis is constant and its volume varies and a "fat tissue of reserve" which almost never disappears except in cases of malnutrition and physiological insufficiency (Markman, 1989). The existing of those two types is determined by metabolic and biochemical differences resulting from the non identical distribution of the  $\beta$ -1 and  $\alpha$ -2 receptors which are responsible for fat tissue sensibility to lipolysis stimuli. For example the  $\alpha$ -2 receptors suppress lipolysis in the pelveotrochanteric region which is exactly the reason for the sometimes observed inability for weight loss in these zones. The latter is also valid for the intraorbital, plantar, palmar and Bichat bulle fat. All those variations in distribution and stability of adipose tissue are of great importance for the therapeutic strategies in autologous fat surgery and will be later thoroughly discussed (5. Factors of influence on the therapeutic results).

### 3. Evolution of autologous fat grafting – Historical overview

The use of fat tissue as a material for soft tissue defects correction or augmentation dates back from the end of 19<sup>th</sup> century. In 1989 E. Billings and J. May (Billings, May, 1989) published a historical overview of autologous fat grafting and in 2004 A. Mojallal divided it into three periods (Mojallal et al., 2004):

- First period – before the invention of lipoaspiration called an "open surgery" period (from 1889 to 1977)
- Second period - after the invention of lipoaspiration called "non refined", "traumatic" (from 1977 to 1994)
- Third period – after the popularization of S.R. Coleman's lipostructure called "refined" (from 1994 to nowadays)

The "open surgery" period represents the en block transplantation of fat tissue without any changes of its structure. The first report on autologous fat grafting was published by G. Neuber in 1893. He described implanting of small quantities of adipose tissue for filling of cicatrix depressions. Neuber reported good postoperative results though he encountered considerable rates of graft resorption in cases of big volumes transplantation (Neuber, 1893).

In the first half of the 20<sup>th</sup> century the method gained a lot of popularity and it was applied in almost all surgical specialties (Billings, May, 1989; Mojallal, Foyatier, 2004). Of course, the technique was widely used in plastic and aesthetic surgery for breast reconstructions and augmentations (Czerny, 1895; Bames, 1953; Schrocher, 1957), deep wrinkles corrections, cheek-bones area augmentation (Lexer, 1910), corrective rhinoplasties (Brunning, 1919) and etc. All authors reported good immediate results and different resorption rates in the late postoperative period which led to a creation of many technical modifications pretending for improving of transplant stability.

The second period is associated with the invention of liposuction in 1977 (Illouz, 1986, 1986, 1988). Initially created to eliminate the unharmonious adipose accumulations the technique turned out an ideal supplier of fat tissue without causing any unnecessary cicatrices. This led to new horizons for autologous fat transplantation. The first one used the unpurified product of liposuction as a transplant was Y. Illouz (Illouz, 1986, 1986, 1988). Later, in 1989 P. Fournier proposed a technique for reinjection of the aspirated fat called liposculpture (lipofilling) but the quoted necessity of hypercorrection and the instability of the results prevented its recognition (Fournier, 1996). In this period, however, many surgeons were applying autologous fat grafting in their practice. This led to a new wave of negative publications, modifications and even an attempt for reviving the idea of the en block transplantation (Ellenbogen, 1986). On the other hand many authors in Europe and USA reported big series of patients with successful application of autologous fat tissue harvested by liposuction and each one of them suggested a personal technique of processing and reinjection of the graft (Asken, 1987; Bircoll, 1987; Bircoll, Novack, 1987; Carraway, Mellow, 1990; Chajhir et al., 1990; Ersek et al., 1998; Illouz, 1986; Fournier, 1996). One of them is S.R. Coleman, with the popularization of whose work starts the third period called "nontraumatic", "refined" (Mojallal et al, 2004). After a profound analysis he summarized the methods and the results of his predecessors and created a new surgical protocol changing utterly the philosophy of the known by this time autologous fat grafting from free transfer of intact adipose tissue to free fat cells transplantation. The method was published in 1994 and called Lipostructure® (Coleman, 1994; 1995; 2001). It is a three staged procedure consisting of:

1. manual lipoaspiration under low pressure
2. three minutes of centrifugation at 3400rpm eliminating the blood, oil and detergents and
3. reinjection in three-dimensional plan

In creating this technique S.R. Coleman pays great attention to the atraumatic handling of adipose tissue which is of paramount importance for autologous fat grafting results improvement. The method was presented and popularized in Europe in 1994. Afterwards, many authors published series of good results after applying Coleman's technique and summarized its advantages and disadvantages (Amar, 1999; Jaufret et al., 2001; Trepsat, 2001; Mojallal, 2003; Laurent et al., 2006). In comparison with the existing fillers the autologous fat transplanted by this technique is an ideal substitute material because of its quantity, autogenous origin and the ability of full integration with the surrounding tissue (fig.1, 2). It is not palpable and the results seem quite natural. The technique is easily applied under general and local anesthesia, more productive than the other methods stated and not harmful for the adipose tissue. According to that, nowadays, lipostructure® is the officially recognized method of autologous fat grafting. Nevertheless one could still find lots of co-existing techniques pretending for equally good results (Ellenbogen, 1986; Teimourian 1986; Guerrerosantos, 1996; 2000; Toledo, 1996). Their technical modifications act in a different way on graft's biology which creates a variety of factors of influence on the postoperative effect. Thus, even though there are irrefutable proofs for the advantages of autologous fat grafting, still there are some controversies regarding its technical implementation.

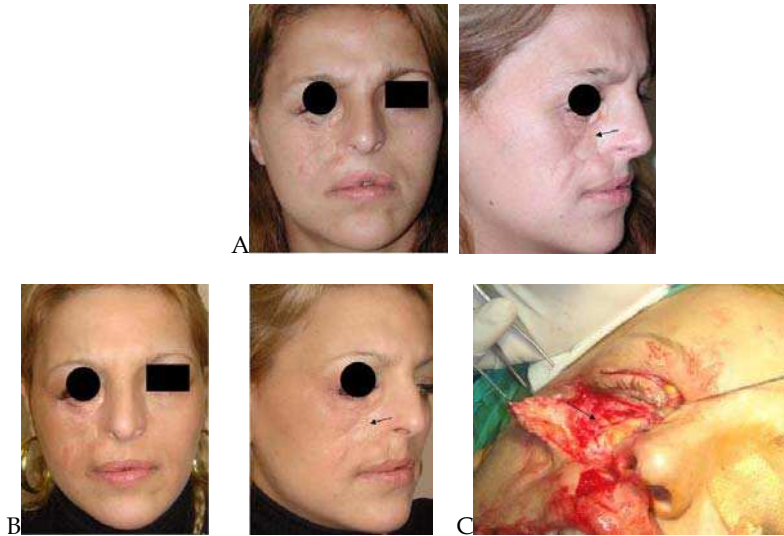


Fig. 1. A patient with cleft №4 according to Tessier classification: A – preoperatively; B – 14,5 months after autologous fat grafting of 3cc in the upper lip and 10cc in right and 7cc in left suborbital region; C – fat tissue found intraoperatively in the recipient site 2 years after the fat grafting



Fig. 2. A patient with bilateral cleft lip and palate, operated according protocol earlier: A – preoperatively; B – 10,5 months after autologous fat grafting of 6cc in the upper lip; C – 12 months after second grafting of 4cc; D – fat tissue found intraoperatively in the recipient site 1 year after the second fat grafting

#### 4. Surgical technique

Nowadays the universal method of autologous fat transplantation is Coleman’s technique “lipostructure” with some variations of its technical implementation. In general it consists of three stages: fat harvesting, specific processing of the aspirate and reimplantation performed in sterile conditions with respect to the fragility of adipocytes.

In our practice fat harvesting is done by deep manual lipoaspiration of the donor site using different in length 2,6mm-inner-diameter blunt cannulas, allowing the free passing of fat cells, attached to a 10-cc syringes of a Luer-lock type. The so described vacuum system is

brought in through a miniature incision placed in the natural creases. The donor site could be any of the subcutaneous depots with or without excessive fat accumulation but we always choose the pelveotrochanteric region for the more appropriate size of the fat cells and their higher activity of lipoprotein lipase (Hudson et al., 1990; Niechajev, Sevcuk, 1994; Fulton et al., 1998). Lipoaspiration is performed under manual regulation of the negative pressure not exceeding 2cc. In the cases operated under general anesthesia no local infiltration is used. For local anesthesia or in the cases of neurosedation between 40cc and 80cc of modified Klein's solution (500ml NaCl, 150mg Lidocain®, 0,5mg Epinephrine®) are applied. Until processing the harvested material is kept in the syringes used for aspiration obturated with sterile caps preventing the harmful contact with air.

The harvested quantity and the choice of anesthesia are individual and depending on patient's character and indications.

The processing of the graft could be done by various methods: centrifugation at different revolutions per minute and durations; decantation; serum washing and so on. In our practice we use centrifugation at 3400 rpm for 3 minutes and serum lavage but we recommend the second method for reasons that will be later discussed.

The graft is centrifuged in the same 10-cc syringes in which it is harvested. The pistons are being removed and the obturated syringes are placed in the centrifuge in sterile metal flasks ensuring sterility. Centrifugation results in sedimentation of material and formation of three layers:

- at the top – an oily layer which is an effect of adipocytes disintegration
- in the middle – the adipose graft
- at the bottom – mainly serum and blood products

This method of processing is considered done when the serum at the bottom and the oil at the top are removed.

The advised by us technique of serum lavage is realized by transferring of the harvested material from the 10-cc syringe in which it was obtained to 20-cc syringe and washing by additionally drawn 10cc of physiological serum. To obtain a fat graft free of oil and blood the saline solution is changed one or two times. The removal of the washing material could be done in two ways according to surgical time: 1) after a short stay in vertical position the saline precipitates at the bottom of the syringe and it is gently pushed by the piston or 2) by pulling up the piston which results in saline leak.

For the next stage the so processed grafts are transferred into 1cc-syringes. Reinjection is done by 1,2mm or 1mm-inner-diameter blunt cannulas which allow structure-safe passing of fat cells and in the same time creates tunnels which diameter do not exceed the critical one for revascularization ( $1,5\pm 0,5$ mm). The combination of cannulas and syringes of these sizes is optimal as they exert very low pressure on the fragile graft and in the same time they are easy for managing. The cannulas are introduced in the recipient site by lots of miniature incisions placed in a manner reassuring the creation of a three-dimensional trellis of grafted tunnels. For prevention of possible embolism reinjection is done while gently pulling backwards the cannula.

Grafted quantities and intraoperative extent of correction is individual and should be planned after a thorough discussion with patients explaining the advantages and

disadvantages of the method, the discomfort of the hypercorrection and the expected resorption. However we do recommend the realization of a slight overcorrection for better results.

The incisions are sutured with Prolene® 6/0 - 7/0. At the end of the operation a light modulating massage of the recipient site and application of epithelotonic unguents is advised. According to localization a bandage or ice compresses in regular basis could be placed for the first 24 hours. At the donor site the wearing of a compressive bandage or garment for a period of 7 days is advised.

## 5. Factors of influence on the therapeutic results

Fat tissue is very fragile and requires a delicate handling. In view of that every stage of the contemporary surgical protocol as well as all related factors have been subjected in time to various investigations aiming an establishment of potential agents of influence on the postoperative results. Hereby we represent a summary on the existing information in literature and our personal contribution by a survey on 148 protocols of autologous fat transplantation realized for a period of 3 years in the Division of plastic and craniofacial surgery, St. George University Hospital, Plovdiv, Bulgaria. The comparison of the investigated factors was realized by application of the described below methods of outcomes assessing (6.Methods of outcomes assessing).

### 5.1 Local anesthetics

There are various reports and different preferences on the optimal type of anesthesia based on quite controversial data on the impact of local anesthetics (Ellenbongen, 1986; Chajhir, Benzaquen, 1989; Ersek, 1998; Har-Shai, 1998; Amar, 1999; Coleman, 2001). In 1995 Jr. Moore published the results of a profound analysis stating no negative influence of the adrenalin on fat cells survival (Moore, 1995). On the contrary, lidocain led to inhibition of glucose transport, lipolysis and growth of adipocytes but the effect was irreversible after cease of contact. Later on, in 2003 the latter was confirmed by J. MacRae's histomorphological research. It found no significant differences between most of the known anesthetics according to their influence on graft viability.

In our practice we always choose local anesthesia when possible - for grafting small quantities or working in limited locations. For the donor site we apply 0,5% lidocain for anesthetizing the place of incision and a tumescent type of infiltration of modified Klein solution (500ml NaCl, 150mg Lidocain®, 0,5mg Epinephrine®) for the rest of the zone. In these cases we prefer a subsequent processing by serum lavage for better purification of the graft. For the recipient site we tend to apply local blocks as the terminal type of local anesthesia leads to tissue edema perplexing the exact planning of the transplant quantity.

In cases with general anesthesia no local infiltration is advised.

### 5.2 Donor site

Any of the subcutaneous adipose tissue depots could be used as a donor site. More frequently these are the places of excessive fat accumulation such as the pelveotrohanteric region (thighs), abdomen, knees, ankles or even chin and hands. According to needed

quantity numerous sites could be used. Nevertheless for treatment of mirror zones (nasolabial folds, cheeks, etc) we advise application of grafts harvested from one place as fat tissue differs according to cells size and activity in different locations (Hudson et al., 1990; Dugail, Ferre, 2002; Casteilla et al, 2004). For example D. Hudson et al. found out that the adipocytes in pelveotrohanteric region are bigger and with higher activity of the lipoprotein lipase. Later on J. Fulton and I. Niechajev proclaimed as optimal for fat harvesting the pelveotrohanteric zone, followed by the abdomen and face region (Hudson et al., 1990; Niechajev, Sevcuk, 1994; Fulton et al., 1998). Many other statements of preferences could be found in literature but we always choose the pelveotrochanteric zone when possible especially in female patients.

### 5.3 Fat harvesting

According to the optimal method of fat harvesting there are many investigations in favor of the manual lipoaspiration (liposuction). This technique combines the advantages of machine liposuction and surgical excision and in the same time it does not have the burden of their disadvantages - respectively adipocytes destruction due to the high negative pressure and disfiguring cicatrices (Novaes et al., 1998; MacRae et al., 2003; Mojallal, Foyatier, 2004). Manual lipoaspiration is noninvasive and as atraumatic towards adipose cells as surgical excision (Moore et al., 1995; Lalikos et al., 1997) which makes it an optimal method for fat harvesting.

### 5.4 Graft processing

The product obtained by fat harvesting consists not only of morphologically preserved adipose structures but also of products of tissue disintegration which sets the need of subsequent specific purification. This stage of the surgical technique is still disputed and there are several known methods of its realization - decantation, filtration, centrifugation and washing with saline solution (Mojallal, Foyatier, 2004; Khater, 2010). Filtration has already been denied for its traumatizing mechanical impact on fat cells and the too long exposition to air (Ersek, 1991; Niechajev, Sevcuk, 1994). The method of decantation is also not preferred for its duration which prolongs surgical time.

Nowadays the techniques of choice for most surgeons are centrifugation and washing with saline solution. 0,9% NaCl is experimentaly proved to be not harmful for fat cells viability which makes the method of serum lavage preferable for many authors (Ersek, 1991; 1998; Horl et al., 1991; Marques et al., 1994; Fulton, 1998; Har-Shai, 1999; MacRae et al., 2003; Smith et al., 2006; Khater et al., 2008). The method of centrifugation is applied in 3000-3400 rpm for 3 minutes. Though there have been many controversial researches concerning its safety, optimal rpm and duration, the technique is proved to be atrauamatical (Jauffret, 1998; MacRae et al., 2003; Smith et al., 2006; Khater et al., 2008).

To investigate the potential influence of the two techniques on surgical outcomes we compared results assessments (subjective and expert) and changes in quantity and morphology of aspirates after their application. From 148 cases of autologous fat transplantation 80,4% were operated with centrifugation of the graft at 3400rpm for 3min. and 19,6% with the already described (4.Surgical technique) technique of serum lavage. At the end of the follow up period the subjective and expert analysis found out considerably



better results in the group with non centrifuged fat grafts ( $p < 0,01$  (fig.3)). The latter proved a dependency of the postoperative effect on the technique of purification in favor of serum lavage (fig.4, 5).

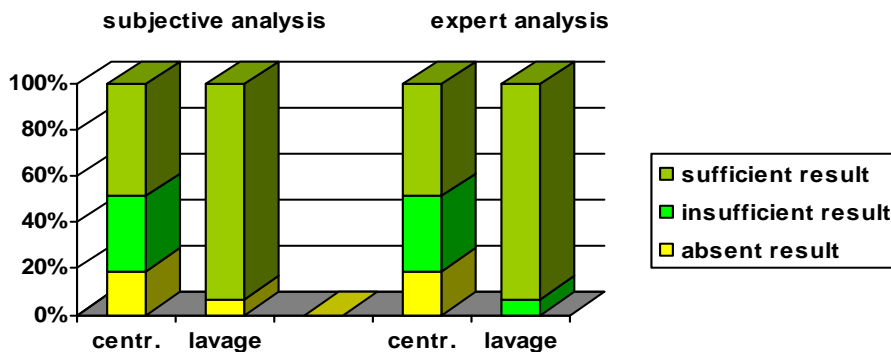


Fig. 3. Assessment of patients and expert jury according to the method of purification: centrifugation (centr.) and serum lavage (lavage).

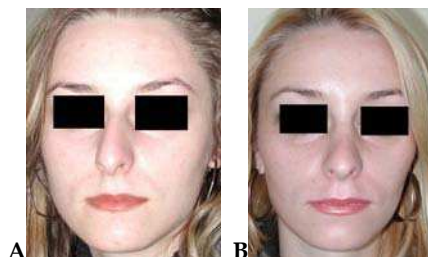


Fig. 4. 26-years old patient with transplantation of 4cc centrifuged autologous fat graft in the upper lip region. A - preoperatively; B - 12 months postoperatively

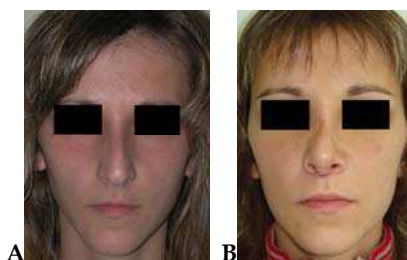


Fig. 5. 24-years old patient with transplantation of 4cc purified by the technique of serum lavage autologous fat graft in the upper lip region. A - preoperatively; B - 12 months postoperatively

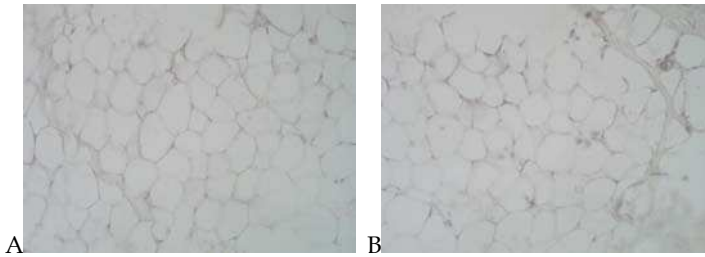


Fig. 6. Syringe aspirated adipose tissue processed by centrifugation (A) and serum lavage (B). Hematoxylin -Eosin staining. Magnification: x200.

Parameters processing type	Average amount before processing	Average amount after processing	Average difference in quantity	T	P
Centrifugation	31,74cc	22,24cc	9,50cc	3,27	<0,01*
Serum lavage	50,72cc	31,75cc	18,98cc		

Table 1. Average difference in aspirate quantity before and after centrifugation and washing with saline solution. \*The difference is statistically significant

Histological analysis found out more preserved tissue elements in the non centrifuged samples (fig.6). Of greatest importance among them was the presence of more immature adipose cells (preadipocytes) which in comparison with adipocytes are more resistant to ischemia, easier for revascularization and capable of proliferation and differentiation. The observed differences in preadipocytes preservation makes the technique of serum lavage more favorable for postoperative outcomes especially in view of recent assumptions that the primary flow of previous techniques was selecting the wrong component of adipose tissue - adipocytes (Scholler et al., 2001).

The quantitative analysis found out that in comparison with saline washing the method of centrifugation preserves two times bigger amount of the harvested material (tbl.1). In view of the worse results and the presence of less tissue elements, the latter makes us think that the method of centrifugation is imperfect according to graft purification by detergents.

The influence of the two methods on grafts viability was studied in vitro in floating tissue cultures and diffusion chambers (Khater et al., 2008). After 7 days of cultivation the morphological analysis again found greater presence of preadipocytes in the non centrifuged samples (fig.7). Among the grafts cultivated in diffusion chambers these results were additionally confirmed (fig.8) by investigation of the immunohistochemical expression of leptin (Alexis Biochemicals; dilution 1:5000) which is a significant marker of adipose cells (Klein et al., 1996; Cinti et al., 1997; Atanassova, Popova, 2000). By analogy, in view of the fact that adipocytes do not have the ability to divide, the immunohistochemical expression of Cyclin D1 (DACO corp.; dilution 1:200) - an universal marker of proliferation (Klein et al., 1996; Cinti et al., 1997) showed higher proliferative activity in the non centrifuged grafts (fig.9). The latter spurs the notion of greater possibility of de novo fat formation in the recipient site after transplantation of adipose tissue processed by serum lavage. This idea

was also supported by the results observed after cultivation in floating tissue cultures (fig.10). In centrifuged grafts weak manifestation of proliferation and differentiation was observed. In comparison, in the non centrifuged grafts could be seen a vast proliferative zone and a zone of distant cell migration containing differentiating preadipocytes.

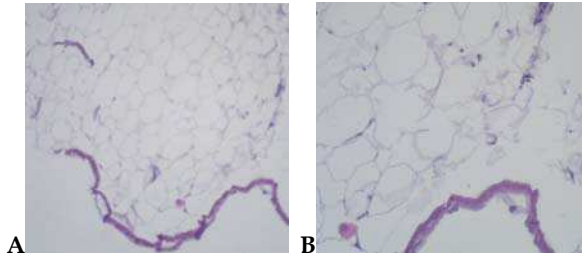


Fig. 7. Diffusion chamber tissue culture, hematoxylin – eosin staining. Magnification x200: A – centrifuged adipose graft culture: presence of adipocytes and a small amount of preadipocytes among them. B – non centrifuged graft culture: presence of adipocytes, preadipocytes and connective tissue fragments containing preadipocytes.

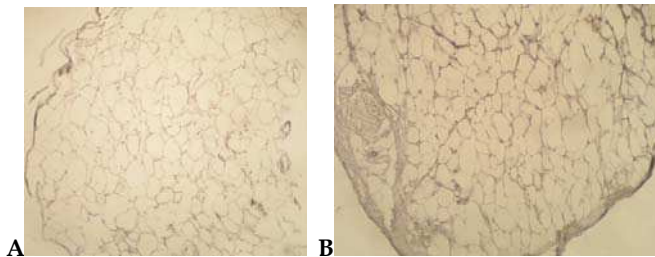


Fig. 8. Immunohistochemical expression of leptin in adipocytes and preadipocytes. Diffusion tissue chambers, magnification – x200. A – centrifuged adipose graft culture – expression of leptin in adipocytes and in small amount of preadipocytes among them. B – non centrifuged graft culture - expression of leptin in adipocytes and in the preadipocytes among them and within the connective tissue fragments.

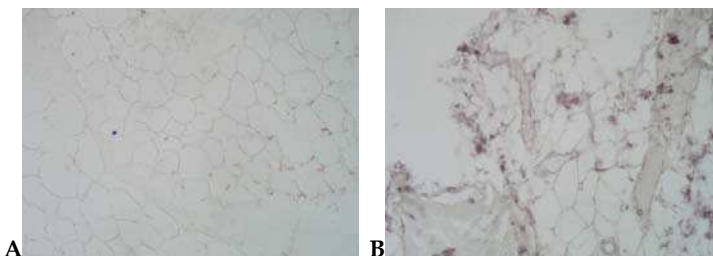


Fig. 9. Immunohistochemical expression of Cyclin D1 in preadipocytes. Diffusion tissue chambers, magnification – x200. A – centrifuged adipose graft culture; B – non centrifuged graft culture

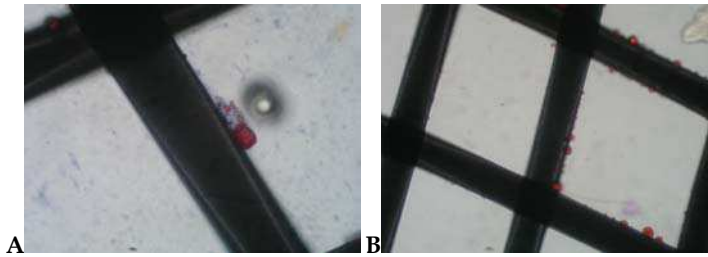


Fig. 10. Floating tissue cultures. Sudan III – hematoxylin staining, magnification x200.

A - centrifuged adipose graft culture: primary zone of cultivation and proliferative zone;

B - non centrifuged graft culture: primary zone of cultivation, proliferative zone and a zone of distant cell migration.

### 5.5 Recipient site

The quality of the recipient site is of great importance for transplant survival. The optimal conditions are to have anatomically and physiologically undamaged tissue structures with good blood supply. It is proved that fat grafting in zones of fibrosis is done under pressure which leads to structure changes in cells and diminishes the chances of survival (Har-Shai, 1999). For optimal results in these cases a certain repetition of the method is needed and after a few applications a considerable improvement of such a zone quality could be observed (Mojallal, Foyatier, 2004).

As good vascularization is of utmost importance for successful transplantation many authors consider the muscle optimal for graft intake because of its blood supply (Nguyen et al., 1990; Niechajev, Sevcuk, 1994; Guerrerosantos, 2000). Though this recipient site still has its supporters (Colic, 1999; Jackson, 2001; Stamos, Xepoulias, 2001) it was proved that it is of a much risk for hematoma formation and fat cells lysis (Mojallal, Foyatier, 2004). Nowadays there is not a unified opinion for the optimal anatomical structure but it is experimentally proved that fat grafting in zones without fat tissue by nature is unsuccessful (Van, Roncari, 1977; 1982).

We investigated the potential influence of recipient site anatomical location on the therapeutic results (Khater, 2010). The comparison of outcomes assessments after grafting in head and neck region, limbs and body found more favorable results with statistical significance ( $p < 0,05$ ) in the head and neck region (fig.11). We attribute the latter to the higher blood supply of this region.

### 5.6 Indications

The method of autologous fat grafting is suitable for soft tissue defects correction or volume augmentation. It is widely used for esthetic indications – esthetic surgery of the face, aging hands corrections etc. There are still some controversies on applying the method for breast augmentation. Though many surgeons think that the technique impedes breast cancer diagnosis by formation of micro calcifications and cicatrices it works quite well in the hands of others if the fat is not directly injected into the gland (Gradinger 1987; Hartrampf 1987; Ousterhout 1987; Khouri et al., 2000; Delay 2009).

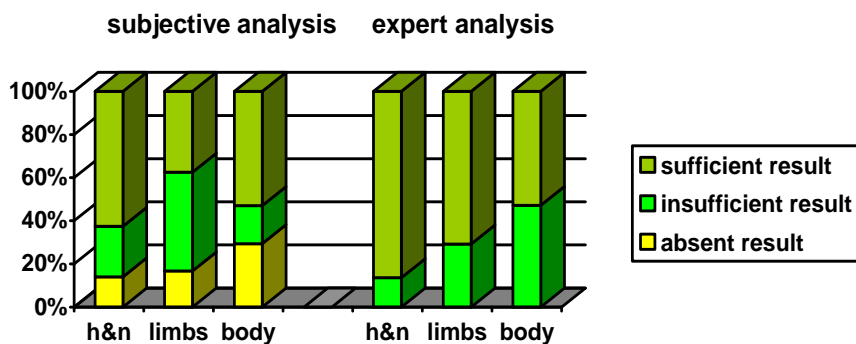


Fig. 11. Assessment of patients and expert jury according to recipient zone location: head and neck (h&n), limbs, body

Initially designed to fulfill the esthetic surgery needs nowadays the method has a broad spectrum of reconstructive indications though there is still a certain lack of distinctness which defect or condition is appropriate for such a surgical solution. Back in 2000 J. Guerrerosantos published a classification of soft tissue defects dividing them into four stages (Guerrerosantos, 2000). The first two groups affect only the subcutaneous tissue and could be treated successfully only by autologous fat transplantation. For the other two stages a combination with other surgical techniques is needed as the defects affect underlying structures in partial or full depth. This classification is very popular and advised for therapeutic strategies creation.

In our practice we compared the results assessments after autologous fat transplantation for esthetic and reconstructive indications and found considerably better results in the esthetic group ( $p < 0,0001$  (fig.12)) probably due to the better qualities and blood supply of recipient sites. In view of that when treating cases with reconstructive indications a planning of multiple applications is advised.

Hereby we represent few cases of example from our practice (fig. 13 - 21).

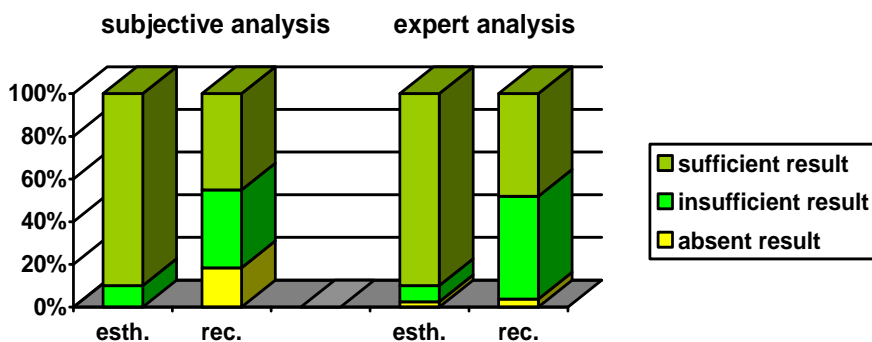


Fig. 12. Assessment of patients and expert jury according to indications: esthetic (esth.) and reconstructive (rec.)

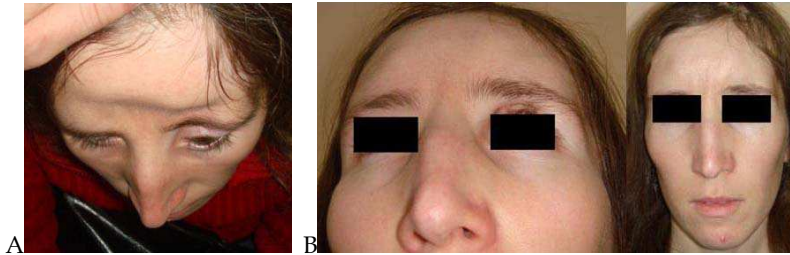


Fig. 13. A 27-year old patient with a forehead contour defect consequence of cranioplasty. A - preoperatively; B - 12 months after transplantation of 25cc purified by the technique of serum lavage autologous fat graft in the forehead region.

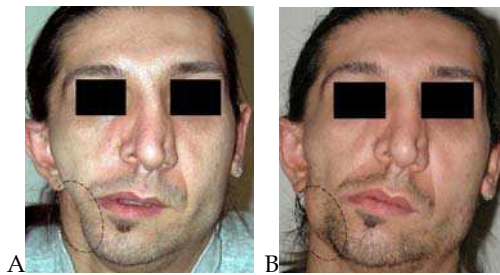


Fig. 14. A 35-year old patient with otomandibular syndrome in the right. A - preoperatively; B - one month after distraction and transplantation of 20cc centrifuged autologous fat graft in right mandibular area.



Fig. 15. A 33-year old patient with contour defect after mentoplasty. A - preoperatively; B - 9 months after 3 applications in 3 months of 16cc non centrifuged autologous fat graft in the chin area.



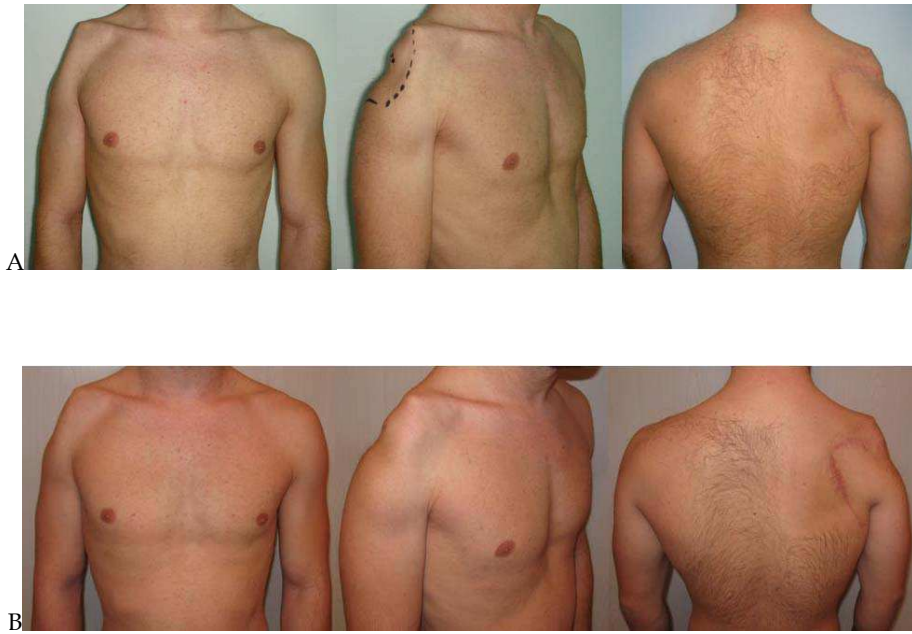


Fig. 16. A 23-year old patient with contour defect after operation for scapular fibrosarcoma 5 years ago. A - preoperatively; B - 3 months after transplantation of 51cc non centrifuged graft in scapular region.



Fig. 17. A 28-year old patient with cleft lip and palate in the right operated in childhood. A- preoperatively; B - 18 months after rhynoplasty and transplantation of 4cc non centrifuged fat graft in the upper lip.

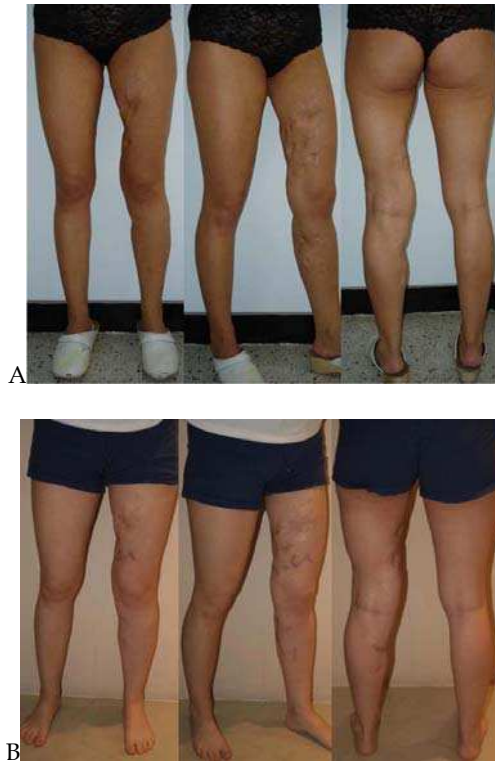


Fig. 18. A 29-year old patient with contour defects of the left leg sequence of clostridial myonecrosis in childhood. A - preoperatively; B - 16 months after transplantation of 100 cc centrifuged fat graft in the different regions.



Fig. 19. A 24-year old patient with lip augmentation and rhynoplasty. A - preoperatively; B - 24 months after 2 applications in 6 months of centrifuged fat graft: 2x2,5cc in the upper and 2x2,5 in the lower lip.



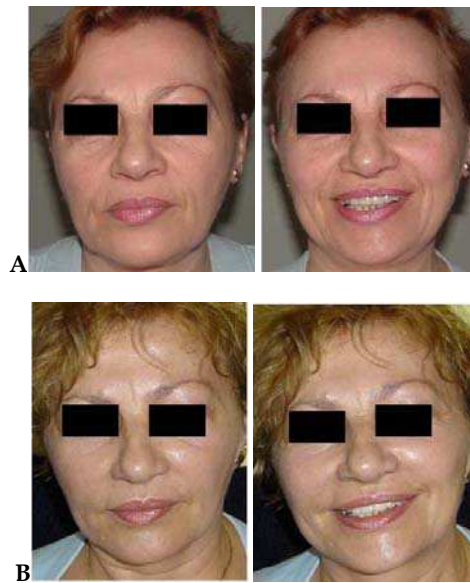


Fig. 20. A 56-year old woman with autologous fat grafting in the nasolabial folds. A - preoperatively; B - 12 months after transplantation of 10cc non centrifuged graft in each fold.

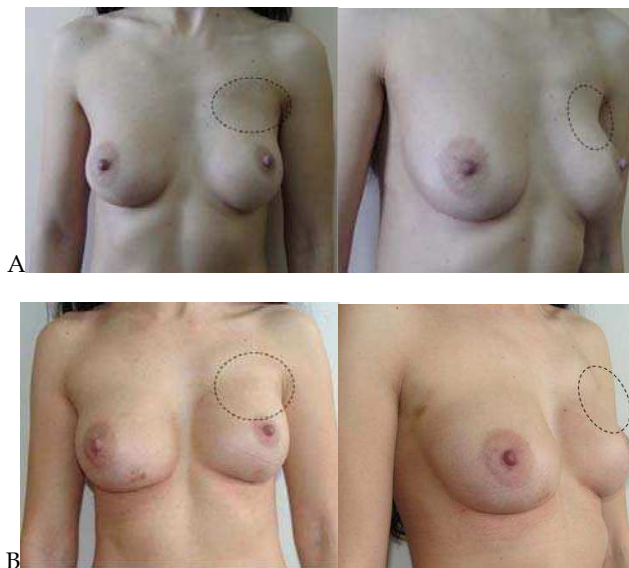


Fig. 21. A 33-year old patient with Poland Syndrome treated with breast augmentation 8 years ago (160cc round prosthesis). A - preoperatively; B - 2 weeks after operative change of prosthesis (180cc round prosthesis) and application of 18cc of non centrifuged fat graft.

## 5.7 Reimplantation

Although there are many stated preferences and created surgical instruments (Chajhir, Benzaquen 1990; Niechajev, Sevcuk, 1994; Toledo 1994; Amar 1999), nowadays the method of choice for fat grafts application is the three-dimensional technique of Coleman (Coleman, 2001). Most of the surgeons use the proceeded material immediately after fat grafting but in literature could be found examples for graft freezing and subsequent use (Coleman WP 1999; Fulton et al., 1998; Bertossi et al., 2000) or graft enrichment with growth factors and other substances increasing the chances of graft survival (Pinski et al., 1992; Scholler et al., 2001).

## 5.8 Intraoperative extent of correction – normo- and hypercorrection

The stated in literature graft survival varies but it is rarely 100%. There is always a certain percentage of transplant resorption. For optimization of postoperative outcomes many authors advise a hypercorrection of the treated zone (Niechajev, Sevcuk, 1994; Amar 1999; Coleman 2001) or three and more applications in the recipient site at regular basis – between 3 and 6 months (Bircoll 1987; Boschert et al., 2002). In our practice the comparison of surgical outcomes assessments showed more favorable results after hypercorrection of the treated zones ( $p < 0,0001$  (fig.22)). There were no statistical differences between multiple and single graft applications.

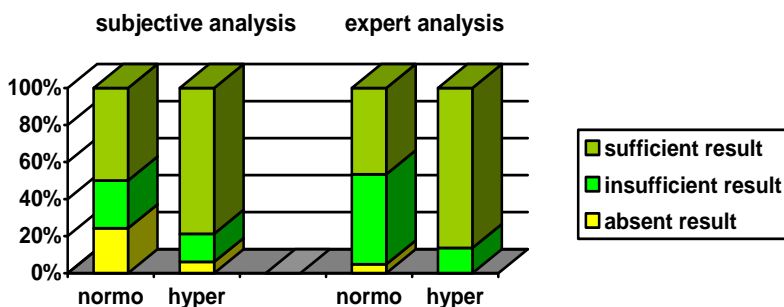


Fig. 22. Assessment of patients and expert jury according to the extent of surgical correction: normocorrection (normo) and hypercorrection (hyper)

## 5.9 Combinations with other interventions

The need of combinations with other surgical interventions is individual and it depends on the treated defect. Nevertheless an immediate combination with another surgical technique in the recipient site is not advised for increasing the chances of traumatism and graft lysis. On the other hand autologous fat grafting with execution of other interventions in adjacent regions such as augmentation of gluteus region with liposuction of thighs or lip augmentation with rhinoplasty was highly assessed by the patients ( $p < 0,0001$  (Fig.23)). The expert analysis showed no significant difference between the groups. These data are not informative for the efficiency of autologous fat transplantation as a surgical method but show that its combination sometimes could improve subjective results apprehension.

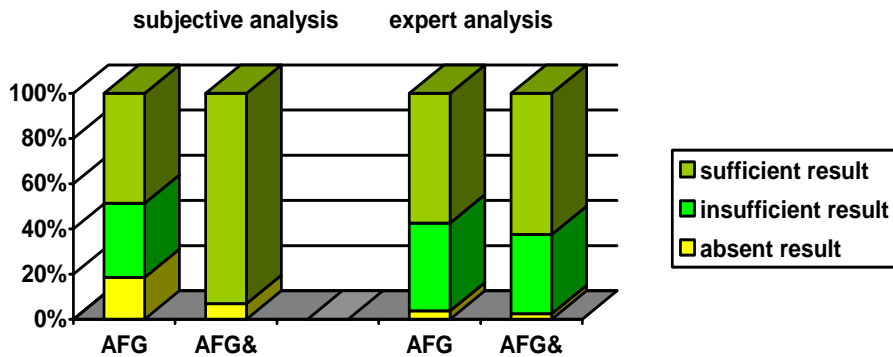


Fig. 23. Assessment of patients and expert jury according to the type of operation: autologous fat grafting (AFG) and autologous fat grafting in combination with other techniques in adjacent areas (AFG&)

## 6. Methods of outcomes assessing

The assessment of the postoperative results is closely related and dependent on the extent of graft survival. In general there are two theories about what happens in the recipient site after fat grafting: the theory of entire cell replacement by the host and the theory of adipocytes survival (Mojallal, 2003). According to the first one, which still has some followers, the induced volume augmentation in the recipient site is due to an entire phagocytosis of grafted cells and accumulation of phagocytes (Chajhir et al., 1990; Nguyen et al., 1990; Eremia, Newman, 2000; Sadick, Hudgins, 2001). The second theory states that the transplanted adipocytes survive and continue their development in the recipient site. Nowadays there are many researches in favor of this statement but the first and the most important one was published back in 1950 by L. Peer (Peer, 1956). The author described an autologous fat transplantation of grafts with different sizes in the rectus abdominis muscle area of 26 patients. The subsequent histological analysis discovered a periphery neovascularization starting at the fourth postoperative day and up to 50% of resorption to the end of the first year, more intensely expressed in the cases with bigger fragments transplantation. These findings were later supported by many experimental, biochemical and histological researches based on biopsies (Carpaneda, Ribeiro, 1993; 1994; Niechajev, Sevcuk, 1994; Jauffret, 1998; Moitra et al., 1998; Gavrilova et al., 2000; Rieck, Schlaak, 2003)

Unfortunately, since then there are not much more means of graft survival or results assessment. After the biopsy of the graft the most informative methods are the magnetic resonance imaging and the 3-D tomodensitometry of the recipient site pre and postoperatively but they are too expensive and not suitable for routine clinical practice (Horl et al., 1991; Liang et al, 1991; Har-Shai, 1999). Echography is not informative. Up to date the only approachable assessment method remains the standardized photo documentation.

After we summarized the experience of other authors we offer a protocol of results evaluation combining all known uninvasive methods. We suggest postoperative outcomes follow up in three stages:

- immediate - to the end of the first week,
- intermediate - between the 3d and 6<sup>th</sup> month, in which the results are still unstable and
- final - after the 9<sup>th</sup> month, after which according to literature the graft is steadily integrated (Horl et al., 1991; Carpaneda, Ribeiro, 1993) and further resorption is unexpected.

The follow up includes standardized photo documentation and filling up of specially designed protocols and patients' inquiries. Photographs should be taken preoperatively and in the three postoperative stages in same and as many as possible projections. We recommend a distance of 1 meter for the face and 2 meters for other regions. The protocols should be filled in by the operating surgeon and should record: personal data, indications, consecution of the intervention, combinations with other interventions, type of anesthesia, donor and recipient site, method of processing, harvested and grafted quantities, intraoperative extent of correction and complications as these two should be as well followed in the 3 postoperative periods. The patients' inquiries are not obligatory but very helpful for evaluating the subjective view of the postoperative period: donor or recipient site pain; any difficulties in social integration due to edema and ecchymoses and most importantly personal assessment of the results as sufficient, insufficient or absent. To avoid the subjectivity some surgeons are proposing a creation of an alternative jury for double blind assessing of the results.

## 7. Complications and disadvantages

Our practice showed that there are rarely any complications after application of autologous fat grafting (fig.24, 25). Among investigated cases complications in donor site were dependant on the aspirated quantity which sets the need for multiple donor sites planning if bigger amount of fat is needed.

Our investigation found out that the postoperative period is not painful in most cases and if it is there is a prevalence of the low intensity rates -  $3,95 \pm 0,29$  in recipient and  $4,22 \pm 0,26$  in donor site according to subjective pain assessment from 1 to 10.

Usually after autologous fat transplantation edema and ecchymoses of donor and recipient sites are observed. As the latter are almost always present they are considered as method's disadvantages. In the investigated cases edema was observed in 65,54% and ecchymoses in 20,95%. According to our patients the disadvantages were present for about one week and in most cases they did not interrupt their habits and social activity (fig.26).

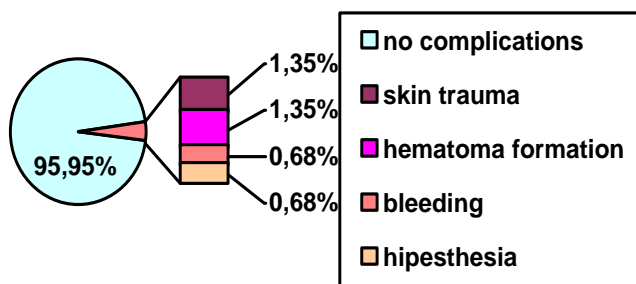


Fig. 24. Postoperative complications in donor site

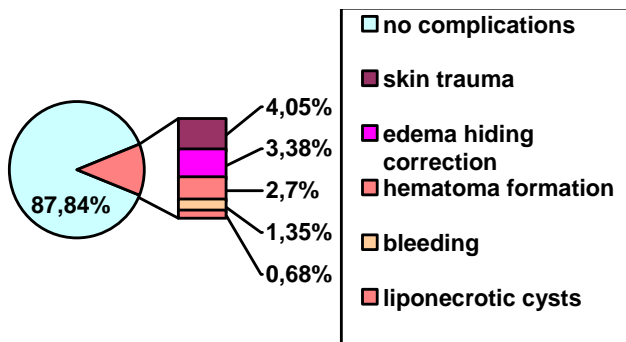


Fig. 25. Postoperative complications in recipient site

In conclusion we could say that the intervention is not painful and rarely leads to complications. It is well accepted by patients and does not require a long period of rehabilitation.

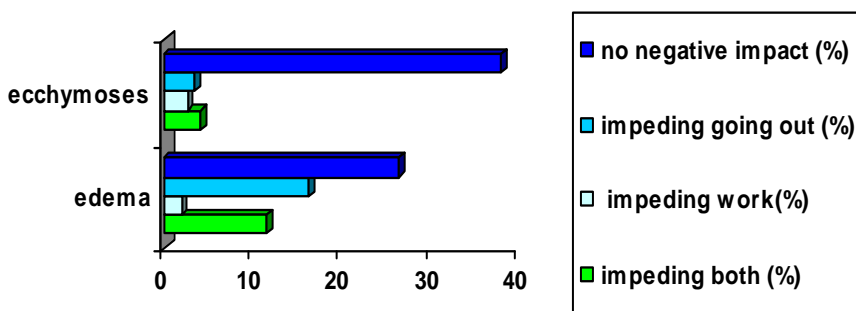


Fig. 26. Edema and ecchymoses impact on social activities. Data by patients' inquiry

Nevertheless there could be some complications due to a bad surgical technique such as:

- Graft migration – when applying big quantities in a cavity or in a manner different from the three-dimensional technique
- Skin irregularities or even necrosis in the cases of too superficial applications
- Liponecrotic cysts formation – when injecting big quantities unable of revascularization
- Infections
- Embolism after fat injection into vessels and so on

## 8. Perspectives

Nowadays, the growing knowledge on the complexity of adipose structure and functions revealed great potentials of influence on the therapeutic results. The contemporary strategies in autologous fat grafting improvement are concentrated on gaining presence of particular tissue structures in the graft (preadipocytes, etc), provoking different substances secretion or creation of methods for transplant enriching and thus improving the chances of its survival and integration.

It is now clear that fat tissue is a major endocrine and paracrine organ producing a variety of hormones and signalling molecules, generally referred to as adipokines (Mohamed-Ali et al., 1998; Fruhbeck et al., 2001; Chaldakov et al., 2003; Trayhurn, Wood, 2004). Among those is the nerve growth factor (NGF), recently proved to be synthesized in the main white adipose tissue depots by the stromo-vascular cell compartment and mature adipocytes (Peeraully et al., 2004; Ryan et al., 2008; Sornelli et al., 2009). NGF is a pentameric protein complex which neurotrophic activities are mediated by the tyrosine kinase A (trkA) - high affinity receptor, specific for NGF and p75 - non-specific, low affinity receptor. Its expression in adipocytes is associated with wound healing and plasticity (Tore et al., 2007) and it exerts metabotropic effect on glucose, lipid and energy homeostasis as well as antilipolytic, lipogenic and angiogenic effects (Emanueli et al., 2002; Hansen-Algensteadt et al., 2006). Moreover, NGF has an indirect effect on fat cells viability, proliferation and differentiation (Peeraully et al., 2004).

In our practice the expression and cellular localization of nerve growth factor and its' receptors - trkA and p75 was investigated in non centrifuged adipose grafts in view of potential possibilities for influence on outcomes. Immunohistochemical investigations demonstrated a moderate positive expression of NGF in both types of adipose cells (mature and immature) and a much stronger expression of the trkA receptor with higher intensity in the preadipocytes (fig.27 - A, B). No expression of p75 was detected.

In future these findings could influence upon the strategies of outcomes improvement as the mechanisms of NGF expression and secretion regulation are already known.

Our investigations also pose some questions on postoperative graft intake. For example, it would be interesting to know to what extent the neurotrophin is involved with postoperative swelling after autologous fat grafting. In 1995 Amman et al. proved that local injection of NGF in rat's paw causes oedema and hyperalgesia (Amman et al., 1995). In the same time the widely used in clinical practice low temperature and glucocorticoids for their anti-edematous effects are known to decrease the neurotrophin's release. Another question which answers would also need further investigations is related to the expected postoperative outcomes after autologous fat grafting in patients with altered levels of NGF as in obesity or type 2 diabetes.

The determination of all those issues could bring to a new era for fat grafting strategies

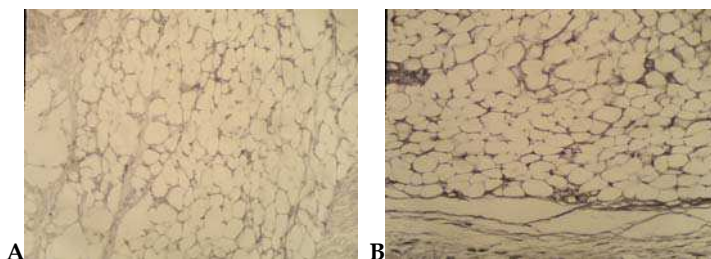


Fig. 27. Immunohistochemical analysis of NGF (A) and trkA (B) in non centrifuged fat grafts - ABC method with primary antibodies (Santa Cruz, USA) in dilution 1:200: A - moderate positive expression of NGF in both types of adipose cells (mature and immature); B - A much stronger expression of the trkA receptor with higher intensity in the preadipocytes

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Plastic surgery continues to be a rapidly growing field in medicine. There have been multiple recent advancements in the field. Specifically, there has been a continuously growing interest in fat grafting, body contouring, minimally invasive surgery, and plastic surgery education. At the same time, there have been continued advances and modifications in surgical techniques, which translate into better and improved results for our patients while increasing safety and efficacy. The title of the book is Current Concepts in Plastic Surgery and, as such, it highlights some of the "hot topics" in recent years. We have invited renowned specialists from around the world to share their valued expertise and experience. Most of the chapters will expose the reader to multiple techniques for achieving desired results, with emphasis on the author's preferred methodology.

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