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ORIGINAL ARTICLE

Water Jet-Assisted Liposuction for Patients with Lipoedema: Histologic and Immunohistologic Analysis of the Aspirates of 30 Lipoedema Patients

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Abstract Lipoedema is a fat distribution disorder causing massive, bilaterally symmetrical enlargement of the lower and in some cases the upper extremities in women. The atraumatic, anatomically appropriate procedure of water jet-assisted liposuction available today represents a promising treatment for these patients who generally suffer from severe subjective and objective impairment. Liposuction treatment can bring long-term improvement if the operative technique focuses on lymph vessel preservation. Immunohistologic analyses show minimal evidence of lymph vessel structures in lipoaspirates. The histologic analysis of the aspirates documents a relatively specific removal ("apheresis") of primarily intact lipocytes with low vascular amount.

Keywords Liposuction · Water jet-assisted liposuction · WAL · Body-jet · Lipoedema · Intact lipocytes · Atraumatic liposuction procedure · Intact lymph vessels

Introduction

Unlike (primary) lymphoedema, lipoedema is characterized by a bilaterally symmetrical, diffuse accumulation of adipose tissue [1]. This disease manifestation, which is mainly limited to the women, is localized primarily to the lower extremities, from the buttocks to the ankle joints, with the thighs and lower legs most affected. The often disfiguring enlargement and painful swelling subjectively impair the patient. Because the torso is not affected, the abnormal fat distribution results in an overall imbalance of body proportions [2–4]. The symptoms of this condition were first described in detail by Allen and Hines in 1940 [5]. It is characterized by orthostatic edema, tenderness, and increased risk of hematoma development. This disproportionate increase in leg circumference in relation to a slender torso cannot be reversed by physical exercise or diet. The course of the disease is progressive (Fig.1).

In the past ten years liposuction has become an established method for treating lipoedema, complementary to conservative treatment options. Liposuction is acknowledged as a possible therapeutic option in the guidelines of the German Society for Phlebology [6]. The aim of therapy is to reduce the circumference and volume of the extremities and remodel the leg contours. However, the first attempts at treating lipoedema with liposuction had adverse results. A worsening of the volume resulted from liposuction procedures in which operators had used a random combination of application directions along both the longitudinal and transverse axes. The unfavorable results occurred as consequences of surgical traumatization, especially to the lymph vessels, which can lead to lipolymphoedema [1]. Cornely [7] observed that "For years there has been a controversial discussion whether the liposuction of lipedema can be carried out without damaging the lymphatic system of the patient. Some authors keep claiming that the liposuction of lipedema is an obsolete method of treatment but this is not true. If the diagnosis of lipedema is undoubted, liposuction with tumescence anesthesia is carried out according to the method described by Klein" [7].

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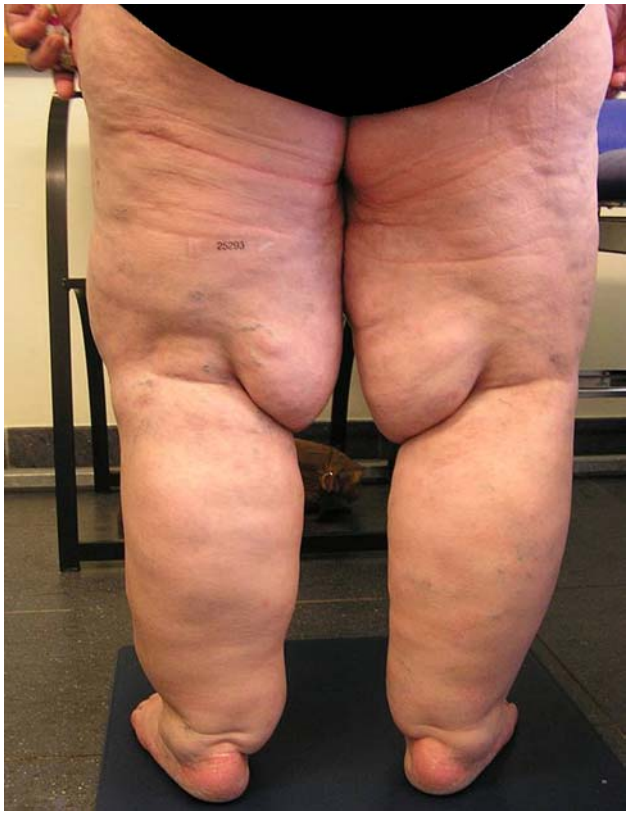

Fig. 1 Lipoedema

Liposuction was reconsidered as a possible treatment for lipoedema after long-term successful results had been observed with anatomically appropriate liposuction methods in which the application was limited exclusively to the longitudinal axis of the extremity. A maximally atraumatic liposuction method was greatly supported by the development of tumescent local anesthesia and the use of thin cannulas [3, 8–10]. Liposuction has since established itself as a therapeutic option among the treatment possibilities for lipoedema [11, 12]. It has also been confirmed that damage to the local lymphatic vascular system must be avoided to the greatest possible extent to ensure long-term improvement of the postoperative results. Orienting analyses performed on avital tissue [13, 14] and the first intravital analyses [15] support a histomorphologic lack of damage to the lymphatic vascular system.

Today water jet-assisted liposuction (WAL) is a treatment method that avoids the disadvantages of the tumescence method, such as volume stress and osmotic effects, without increased traumatic effect.

Aim of the Study

The aim of this study was to conduct a histologic and immunohistologic evaluation of water jet-assisted liposuction (WAL) on a larger group of patients in order to evaluate its effect on lymph vessels, blood vessels, and lipocytes. For a series of 30 patients who underwent consecutive WALs on the inner knees, the entire aspirate for both legs underwent histologic and immunohistologic analyses to evaluate the structural integrity of the lymph vessels. This treatment site was chosen because the ventromedial lymphatic bundle, out of anatomic necessity, is denser in the knee region [16]. The lymphatic collectors run from the back of the foot to the inguinal lymph nodes and the ventromedial bundle extends dorsally behind the medial condyle of the femur. From an anatomical point of view, this area is associated with the highest risk to lymph vessels in liposuction.

Another aim of the study was to verify that WAL can be performed under local anesthesia with tumescent solution and that the normally required firm elastic consistency and high tissue pressure of the treated areas is no longer necessary, and also that the previously required preinfiltration period is obsolete.

Anatomical-Pathologic Considerations

The epifascial lymphatic system of the lower extremities extends as the ventromedial lymphatic bundle, with more than six main lymphatic collectors, from above the malleolus medialis to the inner knee on the medial condyle parallel to the longitudinal axis of the extremity. In the knee area the bundle of lymph collectors lies dorsomedially to the medial femur condyle [17]. Because of the dense bundling of the lymphatic collectors at the medial knee, a region especially exposed in liposuction, there is an increased risk of mechanical injury during treatment. This factor is associated with the risk of operative worsening from lipoedema to lipo-lymphoedema.

On the dorsa of the feet the lymphatic collectors lie above the venous system superficially and are in contact with the dermis-subcutis border here. In all other areas of the leg—including the knee region—the lymphatic collectors run below the venous system at various subcutaneous depths depending on the thickness of the subcutaneous fat. Some collectors have a close spatial relationship to perforating veins. In the thigh region the lymphatic collectors form three levels. In the area of the subinguinal confluence of superficial inguinal veins (Crosse), the superficial inguinal lymph nodes, which are responsible for the drainage of the lower extremities and outer genital region, are closely associated with the outlet of the saphenous vein and may be at risk here in varicose vein surgeries [18].

Lymphoscintigraphic studies of lipoedema patients have shown lymphatic insufficiency without morphologic changes in the lymph vessels as they are found in cases of lymphoedema. Lymphoscintigraphy is therefore a useful method for the clarification of differential diagnoses [19, 20].

The function of the lymph vessels can be understood histomorphologically, or ultrastructurally, in consideration of their mural content of smooth musculature. While lymph vessels with a muscle layer stimulate the flow of lymph using contractile activity, sections of vessels with fragmentary or inadequate mural muscle elements support resorptive activity of the intercellular fluid [21].

Multiple microlymphatic aneurysms of lymph capillaries, especially on the distal extremity, have been described as an anatomical-pathologic characteristic in patients with lipoedema [22].

Typical histologic findings of tissue sections from lipoedema patients show an increase of interstitial fluid with edema of the dermis and septa, an accumulation of mast cells, and a degeneration of adipocytes [23]. A patient who has suffered from lipoedema for years can spontaneously develop functional lymphoedema. This development can be forestalled through early diagnosis and therapy [1].

Diagnosis

The diagnosis of lipoedema is based on clinical findings; in addition, a high-resolution duplex ultrasound [24] is performed. If the clinician has reason to suspect that the patient's lymphatic system has already been affected, an indirect lymphangiography and a lymphoscintigraphy [25] are conducted.

Materials and Methods

Thirty female patients between 21 and 63 years of age with pre-existing pronounced lipoedema (for stages see Table 1) underwent water jet-assisted liposuction (WAL) (body-jet® system, human med AG, Schwerin, Germany) on both legs under standardized conditions with reduced quantities of Klein's [26] solution (1.0-1.5 L) and without bloating of the tissue. The entire aspirate of the inner knees (proximal lower leg and distal thigh) from both legs was histologically and immunohistologically analyzed. The operations were performed using a standardized prodecure. The infiltration was performed in all cases at Range 2 using a body-jet infiltration cannula (diameter = 3.5 mm) until sufficient anesthesia was attained with the infiltration solution. The aspiration procedure was then begun immediately without waiting for fluid infiltration.

In the WAL procedure a fan-shaped water jet is directed at the subcutaneous space in order to separate the adipose cells from the tissue, and at the same time the injected fluid, along with the detached fat cells, is suctioned off mechanically by means of a defined vacuum pressure. For all procedures the irrigation-aspiration cannula (3.5 mm) [16] was directed strictly along the axis of the lymph collectors. After the operation on the first leg, the second extremity underwent the same treatment. The vacuum was set at a constant 0.6-0.8 bar. The quantity of aspirated supernatant ranged from 250 to 2350 ml (Table 1).

Comparison to Tumescent Liposuction Techniques

In tumescent liposuction techniques local anesthesia [9] is frequently used. For this method large quantities of NaCl solution with small amounts of adrenalin and local anesthetic agents (Klein's solution [26] are introduced into the suprafascial space in preparation for the mechanical removal of the adipose tissue. The purpose of this procedure is to "tumesce" (swell) the aspirated area to achieve a tissue consistency comparable to the firm consistency of a watermelon. With this "supertumescence," according to Sattler [9], shearing forces and severe tissue traumatisztion can be avoided. In the tumescent procedure an "infiltration period" of 0.5-1.5 h on average is needed to give the fluid enough time to penetrate into the adipocytes through pressure and osmosis. At the beginning of the infiltration procedure the solution is introduced into the subcutaneous adipose tissue. This solution initially spreads along the connective tissue septa and separates the fat lobules in a process known as hydrodissection. Only then are the adipose cells mechanically removed from the aggregate by means of vacuum pressure. The adipose cells that are aspirated using the tumescent technique have been distended to many times their natural size. Therefore, the aspirate in the suction container is completely different in appearance to that obtained with WAL. In WAL the treated adipose tissue is not bloated and the infiltrating solution is aspirated simultaneously with the adipose tissue; consequently, there appears to be less "supranatant" fat in the aspirate. A comparison may be helpful: In the past, with the tumescent technique between 6 and 10 L [27] of fluid have been used for the infiltration of the front thighs of lipoedema patients in order to achieve the desired firm elastic consistency. When WAL is used, only 1-1.5 l of Klein's solution [26] are required. Therefore, it is not possible to directly compare the supernatants of the different aspirates. The actual quantity would have to be deduced using defined centrifugation.

Immunohistologic Analyses

The critical regions of the inner knee (proximal lower leg and distal thigh) were treated and aspirated separately; the lipoaspirate obtained from the knee area of both legs was also collected separately. The fat-containing operation product floating on the surface of the irrigation solution was skimmed off mechanically for further analysis. Standardized paraffin embedding and histologic

Table 1 Patient data, volume of aspirated fat, and procedure parameters

Patient No.	Age (years)	Size (cm)	Weight (kg)	Fat (%)	BMI	Stage of lipoedema	Waist-to-hip ratio	Aspirate (ml) (supranatant fat)
27196	29	180	65.2	18.8	20	III/1	0.75	250
27219	40	165	79.4	33	25	III/1	0.78	1400
27211	36	157	65	29.8	26	II/1	0.62	1350
25346	40	166	66	35	24	II/1	0.78	500
27390	34	170	71.2	25.5	25	III/1	0.73	600
27215	25	166	73.4	25.3	26.5	II/1	0.72	2000
27319	38	162	87	36.2	33.5	III/1	0.8	1250
27409	36	158	57.2	24.5	22.5	III/1	0.66	650
27463	41	170	70	27.3	24	III/1	0.71	200
27250	36	164	70.4	29.7	26	II/1	0.68	1000
19679	28	164	71.6	30.9	26.5	III/1	0.73	1600
21279	34	172	79	33	26.5	III/1	0.72	1150
27545	23	172	62.8	19.2	21	III/1	0.6	650
26165	63	168	87.4	39.7	31	III/2	0.8	650
26500	38	162	84.4	38.4	32.5	II/1	0.62	2350
27319	38	162	87.2	37.3	33.5	III/1	0.79	1100
10865	36	165	70	30	25.5	III/1	0.69	900
26951	24	161	96.6	30.4	37.5	III/1	0.72	750
27627	37	162	84.4	32.2	32	III/2	0.7	2650
27863	21	174	55.9	17.7	18	III/1	0.78	950
27928	38	174	75.4	28.7	25	III/1	0.63	1500
27925	21	164	80.2	35.6	30	III/1	0.68	1550
27810	23	170	73	25.4	25	III/1	0.68	800
27903	24	174	82.6	30.5	27	III/1	0.68	1300
27187	42	170	72.2	32.9	25	III/1	0.68	1100
10865	36	165	69.2	29	25	III/1	0.68	800
27010	40	163	98.2	39.5	37.5	III/1	0.85	1400
28054	22	175	87.8	34.1	29	III/1	0.68	1100
27600	33	168	63	21	22	III/1	0.69	550
28251	39	161	66.8	29.9	25.7	III/1	0.63	1400

preparation were performed in the laboratory following formalin fixation and centrifugation. The immunohistochemical markers CD31 (vascular endothelium) (DAKO) and D2-40 (selective marker for lymphatic endothelium) (Zytomed, Berlin) were used with the detection system K5005 DAKO alkaline phosphatase red rabbit/mouse (DAKO Cytomation, Hamburg) and chromogen Fast Red. Heat-induced antigen demasking was performed at pH 9.0.

For each specimen, analysis was performed on three step sections stained with conventional hematoxylin & eosin (H&E) as well as one immunohistochemically prepared slide for each. The area of the section examined per slide was 3.0 × 2.0 cm. The analysis was performed independently by two experienced histopathologic examiners. Skin tissue sections exhibiting clear results for both markers were used as positive controls (Figs. 2–4).

Results

The adipose tissue present in variously sized fragments in the aspirate consisted primarily of intact single cells and smaller aggregates of adipocytes which morphologically survived the operative removal from the connective tissue aggregate. Moderate quantities of blood capillaries were consistently detected in each field of study through the expression of CD31. Positive staining with D2-40 antibodies was detected for only two patients, with very few lymphatic lumina (maximum of 1/visual field).

Blood vessels (arterioles, venules, capillaries), with their endothelial lining, show up as oval or ring structures with anti-CD31 antibodies (Fig. 2). When stained with the D2-40 antibody, lymph vessels show up in the tissue section as an unrounded contour or as collapsed endothelial tissue (Fig. 3). In the level of the subcutaneous adipose tissue,

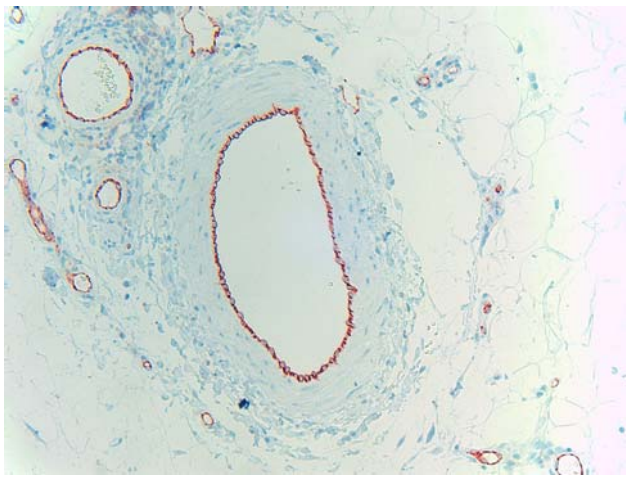

Fig. 2 Controls; CD31 antibody

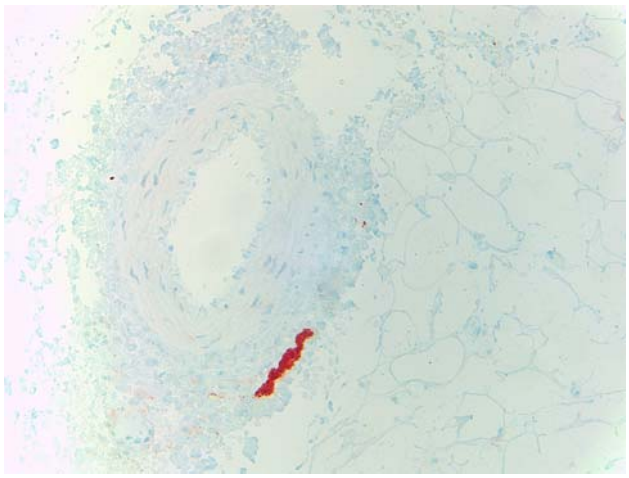

Fig. 3 Controls; D2-40 antibody

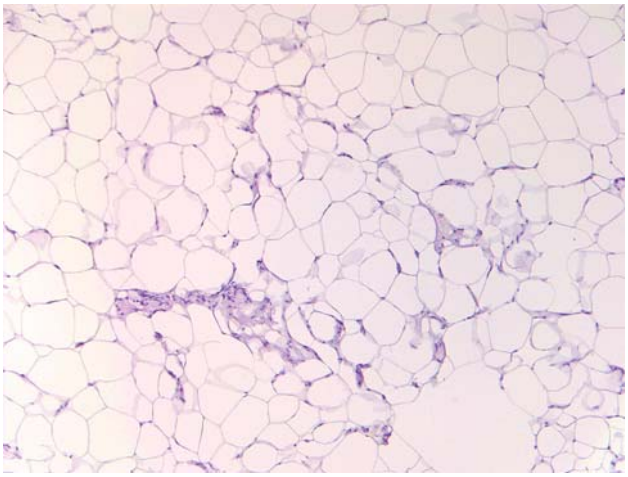

Fig. 4 Controls; lipocyte complexes

blood vessel and lymph vessel tracts pass through the collagen-fibrous septal connective tissue.

The findings for the individual cases, including biographical data and information on disease stage, are presented in Table 2. The evaluated parameters were the histologic overview of the adipoaspirate with regard to the preservation of lipocyte morphology (no evidence of cell membrane rupture) and the immunohistologically imaged density of truncated blood capillaries and lymph vessels in the tissue preparations. The results were classified semi-quantitatively as 0 = not detected, ((+)) = detected in very low levels, (+) = detected in low levels, + = detected, and ++ = detected in high levels.

Slides for evaluation were prepared for all patients. The lipocytes were contained in larger connected complexes (Figs. 4 and 5) or in dispersion to smaller aggregates and single cells (Fig. 6), some accompanied by strands of collagen-fibrous connective tissue (Fig. 5). Dispersion to single cells was generally correlated with a greater degree of single-cell damage in the form of membrane rupture (Figs. 6 and 7). Focal bleeding was found in one case (Fig. 8).

Lipocytes Predominantly Intact

In 28 of the 30 investigated lipoaspirates (patients), the lipocytes were found to be predominantly (> 70%) intact. Two of the 30 investigated adipoaspirates contained, for the most part, separately dissociated adipose cells with distinct signs of destruction and collapse of the cell membrane (Figs. 6 and 7). Immunohistologically, all specimens were shown to contain blood vessels in the images with anti-CD31 antibody (Figs. 7, 9–11). These vessels primarily had the smallest capillary caliber with an average diameter of 0.05-0.1 mm (Figs. 10 and 11). Pieces of venules were found in isolated cases. The number or density of the blood vessel structures ranged from 3 to 20 per microscopic field of vision at medium magnification.

In contrast with the CD31-immunostained vascular images, negative staining results were obtained for the lymphatic endothelial cell marker D2-40 (Figs. 12 and 13). In the sections stained with D2-40, antibody lymph vessels with collapsed walls were found in only two cases: in case 6 focally (Fig. 14) and in case 23, detectable in very low levels (Fig. 15). No intact lymph vessels were detected.

Discussion

The lipoaspirate obtained through liposuction consists of a mixture of subcutaneous tissue components. In the context of tumescent local anesthesia, after initial suprafascial

Table 2 Lipoedema: semiquantitative analysis

No.	Patient No.	Age (years)	Clinical stage of lipoedema	Histology: lipocyte fragments	Immunohistology	
					Blood vessel endothelium = CD31	Lymph vessel endothelium = D2-40
01	27235	40	III/1	+	+	0
02	27390	34	III/1	+	+	0
03	27215	25	II/1	+	+	0
04	27319	38	III/1	(+)	+	0
05	27409	36	III/1	+	+	0
06	27463	41	III/1	++	++	(+)
07	27250	36	II/1	+	+	0
08	19679	28	III/1	+	+	0
09	21279	34	III/1	(+)	(+)	0
10	27545	23	III/1	+	+	0
11	26165	63	III/2	(+)	+	0
12	26500	38	II/1	+	+	0
13	27319	38	III/1	+	(+)	0
14	10865	36	III/1	++	+	0
15	26951	24	III/1	+	+	0
16	27627	37	III/2	(+)	+	0
17	27863	21	III/1	+	+	0
18	27928	38	III/1	(+)	+	0
19	27925	21	III/1	+	+	0
20	27810	23	III/1	+	+	0
21	27903	24	III/1	+	+	0
22	27187	42	III/1	++	+	0
23	10865	36	III/1	+	+	((+))
24	27010	40	III/1	+	+	0
25	28054	22	III/1	(+)	+	0
26	27600	33	III/1	+	+	0
27	28251	39	III/1	+	+	0
28	27296	32	III	(+)	(+)	0
29	28137	36	II/1-2	+	+	0
30	27491	28	III/1	(+)	+	0

0 = not detected; ((+)) = detected in very low levels; (+) = detected in low levels; + = detected; ++ = detected in high levels

hydrodissection, a perilobular infiltration of the adipose tissue lobules is performed, followed by the desired intralobular infiltration. The three-dimensional expansion of the subcutaneous space allows the aspiration of adipose tissues with reduced shearing force, therefore minimizing injury to blood and lymph vessels [10, 12]. In vibration-assisted liposuction, the isolation of adipose cells from the tissue aggregate occurs as a result of the differences in the moments of inertia of the adipose and connective tissues. With the water jet-assisted liposuction method (WAL), adipose cells are mobilized in a comparable manner without causing injury to the vessels. Preservation of the collagen-fibrous septal connective tissue framework creates optimal conditions for postoperative recovery with fibrous tissue retraction [10]. The connective tissue framework also provides channels for both the blood and lymph capillaries. Previous histologic analyses of lipoaspirates were performed primarily to investigate adipose cell integrity [28].

Vessel parts show up as fragments in lipoaspirates, which complicates the task of identifying them reliably using histomorphologic methods. Lymph capillaries have much thinner walls than blood capillaries and are more difficult to identify in tissue sections. Therefore, the special problems presented by the identification of the fragile lymph vessel parts in lipoaspirates and their differentiation from blood capillaries could be anticipated. This challenge can be overcome with immunohistochemical techniques that allow an extremely specific (color) marking of special tissue components.

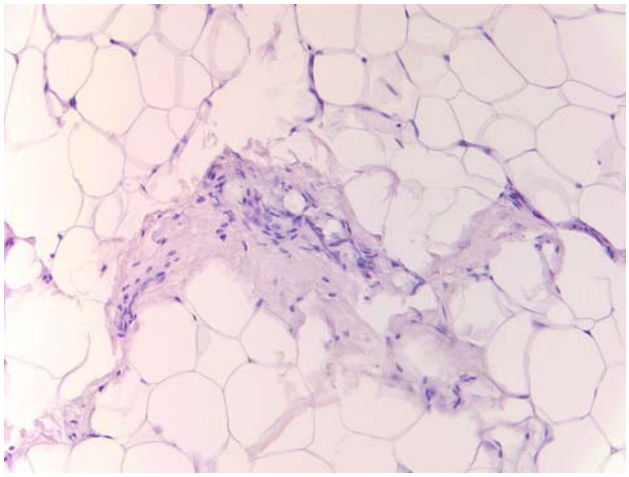

Fig. 5 Lipocyte complexes with strands of collagen-fibrous connective tissue

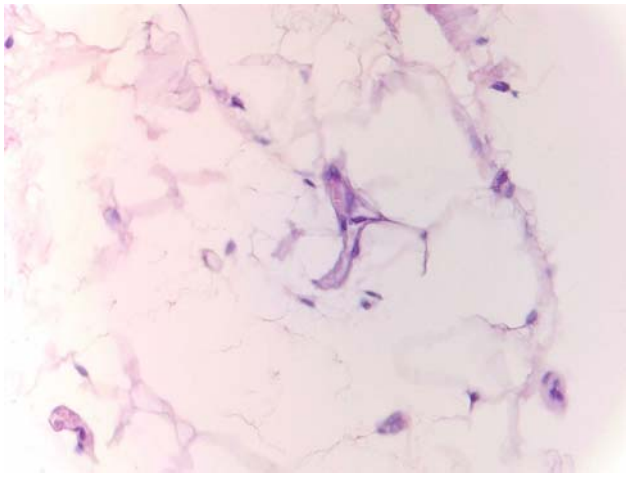

Fig. 6 Lipocytes, dispersion to smaller aggregates and single cells

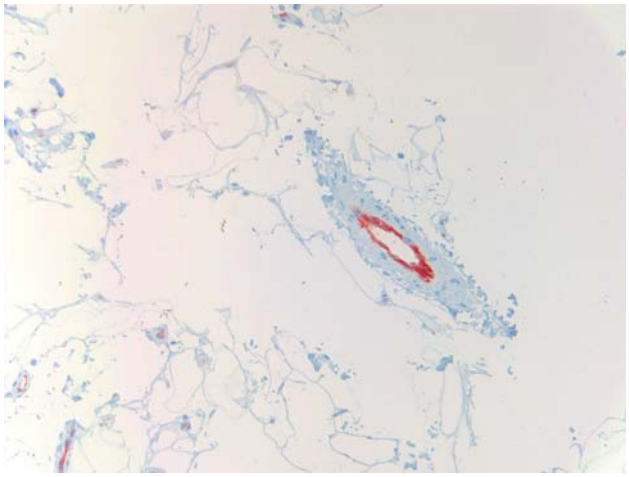

Fig. 7 Blood vessels with anti-CD31 antibody

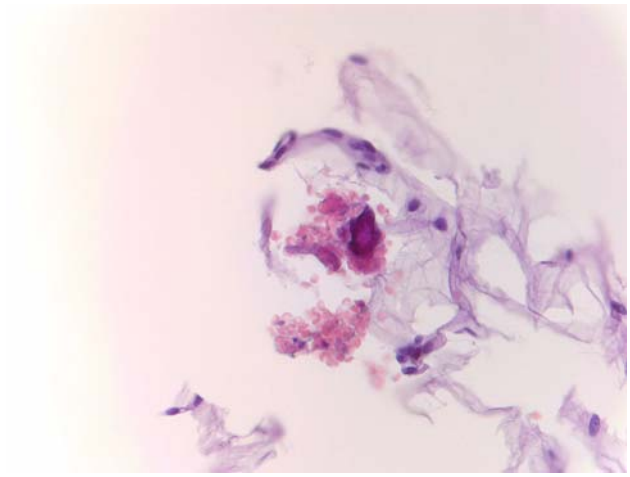

Fig. 8 Focal bleeding in one case

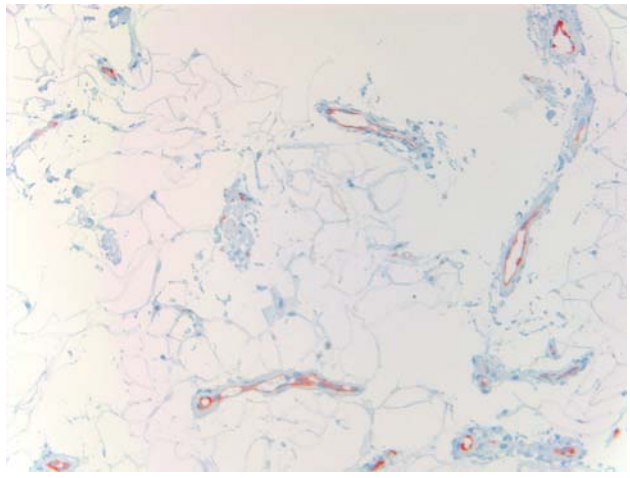

Fig. 9 Blood vessels with anti-CD31 antibody

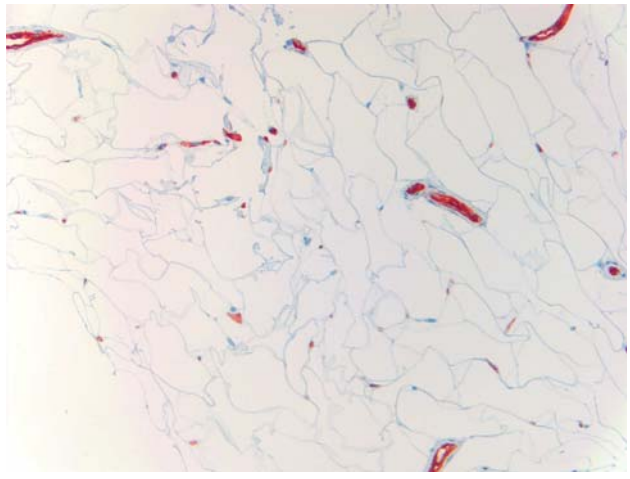

Fig. 10 Blood vessels with anti-CD31 antibody

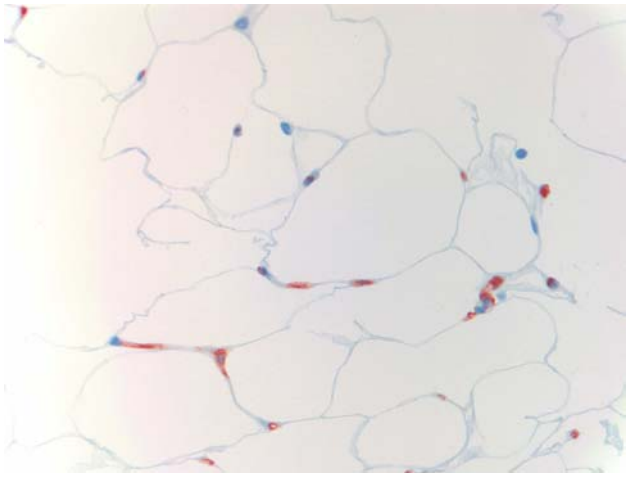

Fig. 11 Blood vessels with anti-CD31 antibody

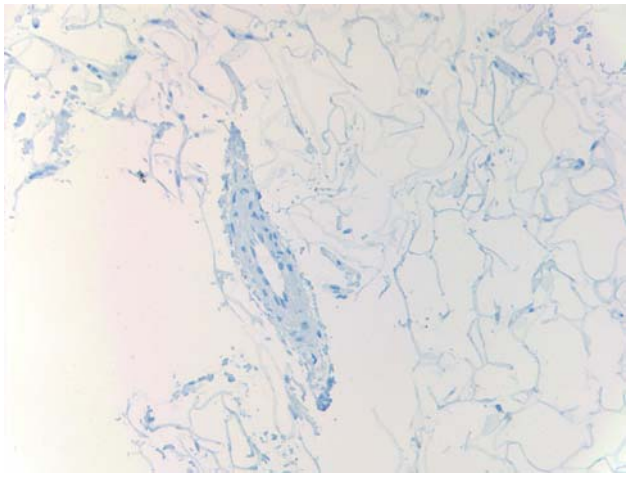

Fig. 12 Negative staining results for the lymphatic endothelial cell marker D2-40

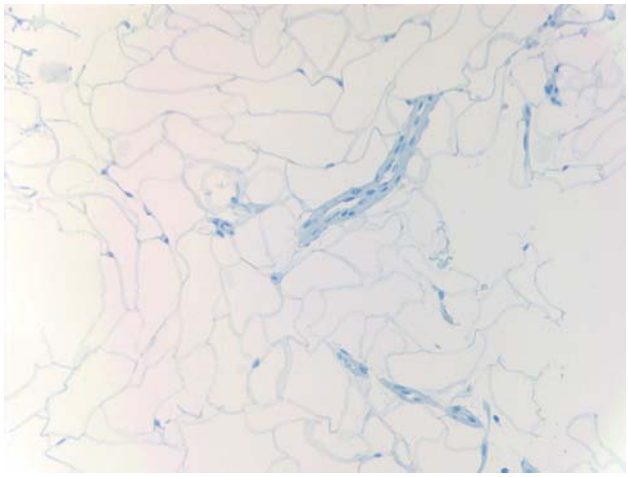

Fig. 13 Negative staining results for the lymphatic endothelial cell marker D2-40

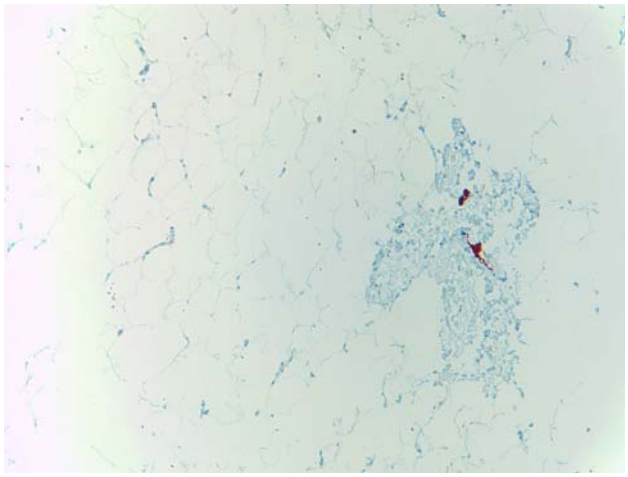

Fig. 14 Lymph vessels with collapsed walls (case 6 focally)

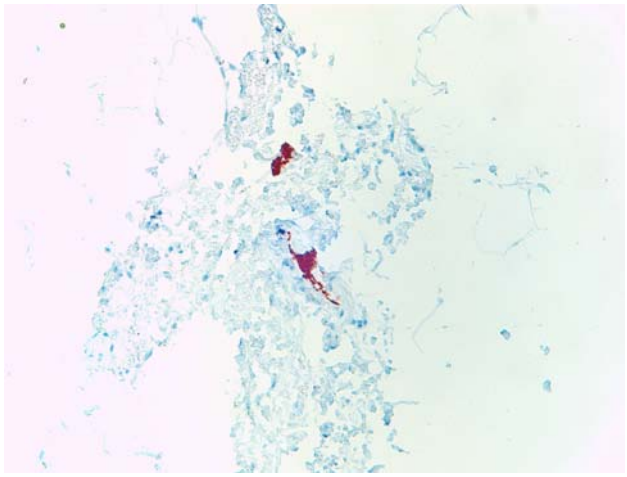

Fig. 15 Lymph vessels with collapsed walls (case 23, detectable in very low levels)

Antibodies to smooth muscle actin, among others, can be used as immunohistologic markers for blood vessels. CD31, on the other hand, is a marker for blood vessel endothelium which does not stain the endothelial lining of lymph capillaries.

The selective representation of lymph vessels has only recently become possible with the introduction of new markers such as the monoclonal antibody D2-40 [29]. Since then, additional, in part closely related antigens have been described as characteristic for lymph vessels and the corresponding antibodies (e.g., anti-podoplanin) introduced for histopathologic diagnostics. The use of the selective lymph vessel marker D2-40 makes it possible to assess whether and to what extent lymph vessels, in complete or fragmented form, are present in the adipoaspirates of patients with lipoedema. A parallel staining with the anti-CD31 antibody, on the other hand, identifies blood-carrying vessels. This method of immunohistologic investigation has already been

demonstrated using histologic preparations from five female patients who had been treated with anatomically appropriate vibration liposuction applied along the longitudinal axis of the extremity under tumescent local anesthesia [15]. According to these results, lymph vessels are practically undetectable in adipoaspirates, while blood capillaries are always present. From these findings it can be concluded that the operative trauma from liposuction causes no relevant damage through the destruction or mobilization of lymph capillaries. This evidence is crucial for the further methodology, practice, and development of liposuction because lymph vessels represent an especially vulnerable and exposed structure in the typical operation site of lipoedema patients.

Summary

Our research has confirmed on a larger number of patients that to a large extent damage to the lymph vessels can be avoided with the use of water jet-assisted liposuction and that this treatment method can produce results that are methodologically equivalent to the tumescence method [15]. The adipose tissue present in variously sized fragments consisted primarily of intact, single cells and smaller aggregates of adipocytes which, for the most part, had morphologically survived the mechanical operative stress. Blood capillaries were consistently detected in moderate quantities per visual field by means of CD31 expression. These vessels were equivalent to blood capillaries in routine staining with hematoxylin & eosin, in part with luminal erythrocytes. Lymph vessels stained with D2-40 were found in only 2 of the 30 cases in our study, and in very small amounts (Table 2). In summary, limited histomorphologic traces of the traumatization of adipose cells and blood capillaries were found in the histologic slides with almost no histologic correlate for lymph vessel injury.

The results suggest that WAL, when applied using anatomically appropriate techniques (working strictly along the longitudinal axis of the extremity), represents a method of treatment without substantially traumatizing the lymphatic vascular system. In comparison with the tumescence method, there are no side effects associated with the water jet method.

Conclusion

The atraumatic, anatomically appropriate procedure of water jet-assisted liposuction (WAL; body-jet®) available today represents a promising treatment for lipoedema patients who generally suffer from severe subjective and objective impairment. Liposuction treatment can bring long-term improvement if the operative technique focuses on lymph vessel preservation. Immunohistologic analyses show minimal evidence of lymph vessel structures in lipoaspirates. The histologic analysis of the aspirates documents a relatively specific removal (“apheresis”) of primarily intact lipocytes with low vascular amount. The analysis of liposuction aspirates from 60 lower extremities obtained from the inner knee area, which represents an especially high-risk region for this type of operation, showed that only minimal or no injury was done to the lymph vessels, if the liposuction procedure was performed strictly parallel to the axis of the lymph collectors.

The immunohistochemical evaluation also confirmed the assumption that a state of tumescence is not required for the WAL procedure thus preserving the structural integrity of lymph vessels. It was also proven that when the WAL technique is used, the preinfiltration period for the tumescent fluid did not have to be observed.

A paradigm shift has thus occurred with the introduction of water jet-assisted liposuction. For this method no tumescence (firm-elastic infiltration condition with high tissue pressure) is necessary. Likewise, no preinfiltration period for the homogenization of the adipose tissue is required. The aspiration procedure is started immediately after the anesthesia has taken effect.

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