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MANUAL LYMPHATIC DRAINAGE WITH SWEAT GLAND DERIVED SIGNET RING CELL CARCINOMA AND FACE LYMPHEDEMA − A CASE REPORT AND LITERATURE REVIEW

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ABSTRACT

Background: Signet ring cell carcinomas (SRCCs) originating from sweat glands are a rare type of neoplasms. Their aggressiveness calls for wide excision margins and lymph node dissection. They typically affect middle-aged men and are often located in the eyelid. Facial lymphedema is often an iatrogenic swelling due to head or neck cancer treatment. It may involve external as well as internal tissues. Complex decongestive therapy (CDT) is a cumulated approach for the management of lymphedemas. It consists of manual lymphatic drainage (MLD), compression, exercises, skin care and patient education. The purpose of this case report is to explore, whether CDT is a suitable treatment for iatrogenic face lymphedemas following signet ring cell carcinoma.

Methods: Case report of a male 51-year-old patient with unilateral left-sided facial lymphedema following multiple surgeries including plastic surgery. The treatment included six MLDs (à 50´), instruction for face muscle exercises, patient education on edema and self-applied massage. The patient declined the application of compression therapy. The parameters measured were pain, sleeping quality, edema, tissue and scar pliability.

Results: Pain was reduced from VAS 5.5 to 1.5. Tissue and scar pliability improved from grade "two" to "one", but did not reach normality. Sleeping quality improved, the overall questionnaire's sum (Pittsburgh Sleeping Quality Index, maximum 21 points) decreased from 13 to 10 points. The swelling of the outer face tissue was not striking or restricted to regions scarcely measurable with common methods.

Conclusions: This is the first case report to reflect CDT in edema following SRCCs. CDT even without compression is an effective treatment modality for minor external and suspected internal lymphedema and scar following oncologic surgery.

Keywords: Face lymphedema, Signet ring cell carcinoma, manual lymphatic drainage

INTRODUCTION

Sweat gland carcinomas are a rare type of malignant neoplasms, representing about 30 % of skin tumors.[1] About 220 cases have been mentioned.[2] Sweat gland carcinomas appear as firm or rubbery red plaques or non-tender subcutaneous lumps.[3] Correct tumor identification is crucial for treatment selection and prognosis.[4] Histopathology and immunohistochemistry are useful techniques for this purpose.[5]

Signet ring cell carcinomas (SRCC) originating from sweat glands are an even more rare type of neoplasms[6] with only 30 reported cases until 2016.[7] The WHO listed the signet ring cell carcinoma under a list of "new entities" in 2018.[8] Signet ring cells can be found in a number of different neoplasms, e.g. basal cell carcinomas or melanomas.[9] A correlation to ultraviolet radiation seems probable and 80 % are located in sun-exposed body regions.[10] Signet ring cells are characterized by a compression of the nucleus to its cellular borders through an accumulation of cytoplasmic substance, therefore mimicking a signet ring.[9] Requena et al.[5] underlined their aggressiveness and called for both wide excision margins and lymph node dissection. The usefulness of chemotherapy remains questionable, whereas radiotherapy is applied in the case of locally advanced tumors.[11] If estrogen receptors - because of the common embryological origin of sweat glands and mammary tissue[12] - or epidermal growth factor receptors are positive, targeted therapies or hormone therapies are also an option.[1]

SRCCs typically affect middle-aged men and are often located in the eyelid. [3, 7] Early diagnosis would be critical for the subsequent therapy choice, but is often delayed because of a diagnostic misinterpretation. As a result, metastasis in the regional lymph nodes can occur.

Facial lymphedema is often an iatrogenic swelling due to head or neck cancer treatment.[13, 14] It may involve external (face soft tissue) as well as internal (e. g. esophagus or trachea) tissues.[15] 75.3 % of

head/neck cancer patients developed lymphedema, thereof 7.4 % externally, 29.6 % internally and 50.8 % developed both.[16] Given the edema site, face lymphedemas are a pathology with grave aesthetic changes.[17] They can lead to physiological and/or psychological consequences[18] such as pain, speech and swallowing problems[16], scar and fibrosis[19], cognitive- and quality of life impairment, depression[13] and social retreat, respectively. Surgery scars have shown to be an obstacle for lymph flow[20, 21] and can therefore be another reason for lymphedema origin.

Complex decongestive therapy (CDT) is a cumulated approach for the management of lymphedemas. It consists of manual lymphatic drainage (MLD), compression therapy, exercises, skin care and patient education.[22] It is largely used after oncologic surgery, such as for breast cancer[23] or cervical cancer.[24] In a small sample MLD and compression therapy showed a significant edema reduction in surgically pre-treated orofacial tumors.[25] The objectives of MLD are to improve lymphangiomotoricity[26], to redirect lymph flow (27) to other non-affected body areas and to contribute to the outgrowing of new lymph vessels.[28] Head and face lymphedemas pose a challenge for therapists because of their common malign origin. Compression therapy in the head/face is also more complicated than in the extremities[13]; the patient's compliance to wear such a device can be compromised.

The purpose of this case report was therefore to explore the question, whether CDT is a suitable treatment for an iatrogenic face lymphedema following a sweat gland derived SRCC.

METHODS

The case of a male 51-year-old patient with unilateral left-sided facial lymphedema following multiple surgeries including plastic surgery is presented. The treatment included six MLDs, instruction for daily patient exercises, patient education on edema/edema prophylaxis and self-applied massage for edema re-direction. The patient declined the application of compression therapy, so this was omitted. The following outcome parameters were collected before/after each therapy and at the beginning/end, respectively: pain (visual analogue scale 0-10), sleeping quality (Pittsburgh Sleeping Quality Index, PSQI, German version; max. 21 points, the higher the worse)[29], edema status (face images) and tissue and scar quality (palpation, 3-point Likert scale: "0" = normal tissue, good pliability; "1" = tissue firm elastic, pliability possible, but reduced; "2" = tissue hard elastic, pliability hardly possible). Further questioning on eventual particularities from the patient's point of view during the prior week were taken at the beginning of every session. The patient gave informed written consent for using the data gathered. This project was approved by the ethics committee of the University of Applied Sciences Upper Austria, Nr. A-2020-065. This case report followed the CARE directions for case reports.

PATIENT´S HISTORY AND ANAMNESIS

In late autumn 2015 the patient first recognized a small lump on his left cheek. In 2016 this was classified as an aberrant sebaceous gland. After several months and notified growth, the patient sought a second opinion. Meanwhile the lacrimal sac had become edematous. An ultrasound brought a diagnosis of inflammation and sinusitis. Again, many months passed, only the third opinion referred the patient to a MRI scan and a tissue sample excision. This confirmed the diagnosis of a sweat gland derived SRCC, staged as pT3bN0M0. Further assessments (total body scanning, thoracic, axillary, mediastinal, abdominal, retroperitoneal, iliacal, inguinal lymph node assessment) did not reveal further pathologies.

The patient underwent surgical excision three times. The first time on November 14th 2018. The surgical margins still contained tumor tissue in microscopic histopathology. A second excision followed one week later, but also failed to produce clear margins. Only the third surgery two weeks later was successful. This comprehensive attempt left the patient with a large left-sided cheek- and nose defect area. The wound was closed primarily with Epigard, a temporary synthetic skin dressing. This was corrected by plastic surgery in December 2018. A forehead flap was mobilized and moved downwards close to the inner eye angle to cover the defect and shape a new lower lid. Because of the weak scientific evidence for chemotherapy, the tumor-free lymph nodes and the lack of distant metastasis, chemo/radiotherapy were not pursued. A second plastic surgery in November 2019 harvested a retro-auricular skin flap, transplanting it to the lower eyelid. A CT scan and a tissue sample from the medial eye angle were inconspicuous. The scan also revealed scar tissue in the former tumor bed and the eye region. The patient was prescribed Zoldem (0-0-1/2), Citalopram (1-0-0) and Novalgin, the last to be taken at his own discretion. He was instructed to avoid sun exposure for at least one year and to avoid lifting/carrying heavy objects. He should self-massage the scar after complete healing and keep his upper body upright. The patient had a follow-up examination every three months with a visual inspection, palpation and a PET scan every other time.

In the weeks and months after the second plastic surgery the patient noticed increased swelling, especially in the morning. He observed escalating tissue change in his upper eyelid, this becoming tighter and firmer. In January 2020 a left upper molar had to be removed. Following the dentist´s therapy, an ever-increasing pain up to VAS 6 started. He also complained about breathing problems through his left nostril. He had a kind of closed sensation in his left ear. The patient was prescribed Vitapos eye jelly ointment to support the

lacrimal production and to keep the eye surface moist. He got Bepanthen scar jelly to care for and mobilize the scar five minutes daily and sleeping pills for his heavily troubled sleeping. He was prescribed a watch glass bandage which he applied for several weeks, which did not change his eye complaints. Mastication was a bit difficult and had to be performed carefully to avoid self-biting in the cheek/lip because of a sensitivity disorder in the inner mouth. The patient was furthermore suffering from cardiac arrhythmias and stress-induced somatic problems. The last inconspicuous oncologic follow-up before starting the CDT was two weeks earlier. An acquaintance, a physiotherapist, but without MLD expertise, initiated the contact to the MLD therapist.

The patient is married with two children and financially independent. The patient led a sporty life-style until the surgeries with a BMI of 25. His family (wife, children, siblings and parents) and his colleagues offered continuous support. He was able to resume his profession (IT specialist in lower management position) after the sick leave. A special arrangement provides him with another 20 days of vacation each year.

INTERVENTION

The patient was treated six times once a week, for 50 minutes a time, starting in May 2020. Treatment started with the patient sitting in front of the treatment couch with his head resting on his hands and a pillow, respectively. This position facilitated the start of the neck treatment (Profundus-Terminus and Occiput-Terminus). Treatment continued by activating the axillary lymph nodes with "stationary circles" in the arm and thoracic region. The back of the head, the neck and the deltoid region was treated according to the Dr. Vodder-method with "stationary circles" and "pumps". Afterwards the patient lay supine, head elevated. The axillary lymph nodes were treated once more, followed by stationary circles on the lower mandibular region, the so-called "fork grip" - anterior and posterior of the ear continued by the face- and intensive scar treatment. The accumulated lymph fluid on the left side was directed from the eye region through the cheek and chin to the ventral neck and shoulder and the axillary lymph nodes. The multiple scars (cp. figure 2) were treated with stationary circles, thumb circles and "soldiers" (stationary circles with steep fingertips. The latter were applied before treating the nose (cheek scar) and once again before treating the forehead (forehead scar). Special eye grips were added (lower eyelid, upper eyelid and orbital space) and supplemented by so-called index-finger circles. The inner mouth drainage followed the face treatment completed by a cheek repetition with stationary circles from the outside. The face's right unswollen side was treated normally according to the Dr. Vodder-method.

The patient was shown exercises to be done at home: first self-applied massage with both hands from the left-sided face over the middle sagittal face line on the upper/lower chin, cheek, lower eye part and upper eye part leaving the eye in the middle of two fingers and the forehead with lateral direction strokes, subsequently down to the neck and axilla. He was advised to apply this routine daily for at least 5 minutes. Secondly, he was instructed to do several face muscle and mimic daily exercises focusing on the mouth and eye parts (inflate cheeks, tip mouth, close/open eyes firmly and furrow/lift brows) with 15 and later 20 repetitions each, two series with a break of 45 seconds in between. The exercise time was left to the patient's discretion, preferably when he felt displeasure (usually during work), a re-filling of the eye region or troubled sleep because of pain and/or swelling.

Because of the COVID-19 pandemic, both the patient and the therapist wore FFP2 masks. The patient's mask was removed when reaching the cheeks, otherwise a thorough face and scar therapy would not have been possible. The therapist wore gloves throughout and double gloves for the inner mouth drainage.

RESULTS

Figure 1 shows the results of the pain intensity score and the 3-point-Likert-scale based tissue- and scar pliability, respectively. Pain was constantly reduced from the first session

(5.5) to 1.5 (last session). Tissue- and scar pliability improved from grade "two" to "one", but did not reach normality.

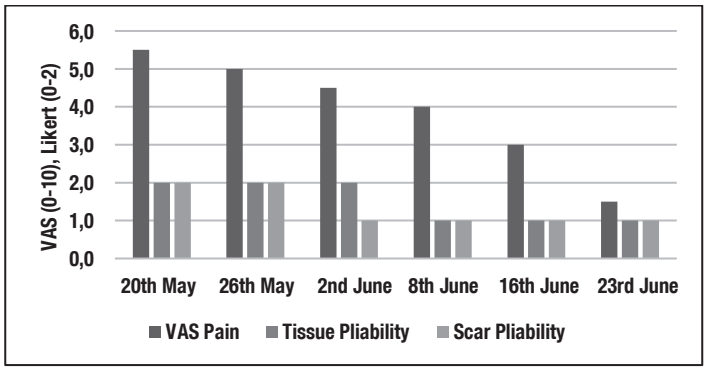

Figure 1: Pain score on VAS, tissue pliability and scar pliability on 3-point Likert scale

Table 1 shows the results of the PSQI and Figure 2 shows the patient's face at the starting point. The PSQI scored 13 points at the beginning and 10 points at the end. The PSQI results are weighted points according to the scoring instruction.

Question	Start				End			
During the last month, When have you usually gone to bed	22:30				22:30			
How long (in minutes) has it taken you to fall asleep	10-20 min				30 min			
What time have you usually gotten up in the morning	07:00				07:15			
How many hours of actual sleep did you get at night	08: 00				08: 30			
During the past month, how often have you had trouble sleeping because you	0	1	2	3	0	1	2	3
Cannot get to sleep within 30 minutes				x			x	
Wake up in the middle of the night or early morning				x		x		
Have to get up to use the bathroom	x				x			
Cannot breath comfortably	x				x			
Cough or snore loudly	x				x			
Feel too cold	x				x			
Feel too hot	x				x			
Have bad dreams	x				x			
Have pain				x		x		
Other reason(s), pl. describe	x				x			
During the past month	0	1	2	3	0	1	2	3
How would you rate your sleep quality overall ‡			x			x		
How often have you taken medicine (prescribed or over the counter) to help you sleep				x				x
How often have you had trouble staying awake while driving, eating meals, or engaging in social activity	x				x			
How much of a problem has it been for you to keep up enthusiasm to get things done			x				x	

Table 1: PSQI results start/end. Key: 0 = not during the past month, 1 = less than once a week, 2 = once or twice a week, 3 = three or more times a week; ‡: 0 = very good, 1 = fairly good, 2 = fairly bad, 3 = very bad

Figure 2

Narrative results: the patient reported pain as usual and increasing nocturnal swelling after the first treatment for three nights, followed by the first two sound sleeps through the whole night for months. Afterwards the situation worsened again, but did not reach the same peak as before. After the second therapy the patient slept two nights soundly without interruption. He reported no longer being awakened by the pain. He was able to remain in a slumber like state and resume deep sleep. He reported a sleeping position dependent pain: the left lateral recumbent was much better than the right lateral recumbent. There was no change in sleep after the third therapy. The scar was somewhat itchy and stung. The situations, in which he totally forgot about his condition during work hours and leisure time increased, moving the problem into the background. After the fourth therapy the patient was slightly disappointed, because of hoping that the pain would regress to zero level, which was not the case. His sleeping behavior was similar to the week before. At the beginning of the sixth and final therapy session the patient reported that he had slept soundly every night, with pain of VAS 3 in the morning, which dropped to 1.5 during the day. He was able to concentrate better on work tasks and other activities, giving him more opportunities to live a normal life. The cheek became more flexible. The mastication problems subsided completely. Two and a half weeks after the final session a telephone follow-up revealed that the pain/ sleeping had been stable for a fortnight, then a slight deterioration was observed. The patient had stopped taking his sleeping pills.

DISCUSSION

In this case report a patient with a presumably uttermost inner tissue face edema was treated with MLD six times, for 50 minutes a time. In this section the therapeutic efforts and results are discussed, set into a physiologic frame of tissue healing and compared to other scientific findings.

Post-surgery comorbidities

The patient's correct diagnosis was delayed for months, even years, giving the tumor time to grow. In comparable head and neck basal cell carcinomas, the tumor volume doubling time was 150 days.[30] In suspicious cases patients should be referred early for excisional biopsy and specialists to avoid any loss of precious time or diagnostic pitfalls.[31, 32] The delay and subsequent more invasive surgery led to an expansion of the excision volume, substantial tissue damage and a disfiguring scar. In a small sample Hachul Moreno et al.[33] proved with lymph scintigraphy in lower limbs, how substantial surgery can alter the physiologic lymph flow. There is no reason to believe why head tissue should react otherwise. A procedure of ongoing surgeries is not unusual in skin tumors[34] with a rate of re-excision of up to 30 %.[35] The later authors referred to a difficulty in balancing extensive

invasive treatment with high post-operative morbidity. As SRCCS are highly aggressive[5], there was no other solution when trying to establish tumor-free margins.[36]

Scar and edema formation

Despite the lack of lymph node dissection or radiation[13], this patient developed an edema. Scar tissue is suspected to be the underlying reason[20] as it is of low quality, with the fibers arranged haphazardly[37], therefore hindering lymphatic drainage.[38] The patient's tissue sample showed hyalinized connective tissue. A hyaline degeneration comes with fiber destruction, leading to a protein lumping. It is noteworthy that keloid formation is characterized by thickened hyalinized haphazard collagen bundles, which can obliterate their surroundings.[39] Both, hypertrophic scars and keloids bear some histological features in common.[40] At least a hypertrophic scar, if not some sort of keloid, although not visible on the outside could have hindered the patient's lymph flow. Warren and Slavin[21] assessed 11 patients with swelling in conjunction with scars with lymph scintigraphy. In 73 % it was not possible to depict any lymphatic drainage bridging the scar. This pathology does not improve even in months or years.[20] Huang and Ogawa[41] also mentioned stress to be a systemic factor contributing to pathologic scar formation. Stress was documented in this very patient.

The role of the Epigard wound dressing must be reflected critically in view of pathological scarring. The extensive wound surface, which needed Epigard to be changed daily could have promoted stretching and mechanical forces, which are known to contribute to scar contracture.[42] Santos et al. described the formation of a matrix network with Epigard dressed wounds in a three-arm animal trial, surrounded by lots of inflammatory cells and fibroblasts. Underneath they found a significant layer of collagen fibers. They concluded that a normal healing cascade did not set in.[43] This leaves the question if Epigard can potentially contribute to abnormal healing and subsequently to scar formation.

Increased scar formation can also be due to other mechanical forces.[44] In the face involuntary eye movements and mouth movements during speaking or eating are unavoidable. Hypertrophic scars can eventually expand to the underlying tissue and even muscles, ending in a restricted range of motion and impairment of daily activities.[45] Scars can be treated with MLD, a technique of soft tissue mobilization, which can accelerate the healing process.[46] Queija et al.[47] put emphasis on the fact that swelling also leads to the compression of neighboring structures. It is noteworthy that this patient had problems with hearing, breathing and mastication.

MLD application

Given the oncologic nature of the edema a prescription of six therapy sessions is on the lower margin. A loosening of fibrotic scar tissue and the outgrow of new lymphatic vessels take time. A prompt referral to lymphedema treatment would have been strongly advised[18] in this case. A qualitative study showed that patients would undertake great efforts, only to improve their condition.[19] Lymphedema is a disease often neglected and overlooked by medical practitioners, but must be strongly suspected after invasive oncologic surgery. Better lymphedema knowledge would ensure earlier treatment and save patients from suffering.

MLD is effective with facial lymphedemas after oncological treatment.[48, 17] It provides benefits such as significant decreased swelling[25], enhanced range of motion and pain relief.[19] Piso et al. [25] successfully used MLD and compression garments for head edemas. Compression therapy is also deployed for different scars. MLD could alleviate the patient's scar complaints and pain in this case report. However, he declined the use of head/face compression. It can be speculated, that this would have enhanced the outcome.

Lymphangiogenesis is a contributing factor for edema resolution. Swartz et al.[49] described how an organized interstitial fluid flow contributes to lymphangiogenesis. Lymphatic endothelial cells are susceptible to cell-stretch and shear stress[50], releasing a Vascular Endothelial Growth Factor (VEGFc). VEGF is a prolymphangiogenic protein promoting the growth of mature lymphatic vessels. Although VEGFc contributed to the resolution of a skin edema in a rodent model[51], it remains unclear whether human skin would react in a similar way. Uzarski et al.[51] concluded that the lymphedema was also resolved because the fluid had bypassed the artificial obstruction through the neighboring interstitium. The bypassing becomes clear, when taking pre-lymphatic channels into consideration, seeing that they are some sort of tissue channels leading the interstitial fluid towards the initial lymphatics. A direct connection between pre-lymphatic channels and lymphatic vessels was proven by Asioli et al.[52]

Pain and sleeping quality

The patient's pain level, which started at VAS 5.5 was reduced to 1.5 during the treatment, presenting an enhancement of 4 points or 72.7 %. As there is no minimal clinically important difference reported for pain of this very source, a change of 10 % was considered relevant. Given the impact of chronic orofacial pain on quality of life[53] this was deemed a success. Chronic orofacial pain is a substantial factor in reducing the health-related quality of life [53] and remains a substantial problem even years after surgery.[54] The patient's pain improvement enhanced his quality of life during day hours, as well as his sleeping quality. Interestingly, the patient reported increasing pain and edema accumulation when sleeping on his right side. This can be explained by the scar and the watersheds, respectively. Normally various lymph vessels would cross the watershed (i.e., median line). Scar tissue could have prevented those normal anastomoses to the contralateral lymph-territory, whereas gravity and a pillow would have facilitated lymph flow, when lying on his left side. Sleeping quality in the PSQI improved by 3 points, but was nevertheless

troubled when considering the sum of 10 points at the end. Only a result of 4 points or lower would have indicated normal sleep. However, these 10 points were achieved without sleeping pills.

The swelling of the outer face tissue was not striking or restricted to regions scarcely measurable with common methods, so face measurements have not been presented. Instead, emphasis was put on outcome measures such as pain, sleeping quality and patient-related outcome measures in a qualitative narrative manner. The supposed internal lymphedema could have been depicted by a CT or MRI[47], which was not possible in the extramural practice setting.

LIMITATION

No patient reported outcome measure assessment was used to assess the patient's satisfaction. It must be noted, that available questionnaires only assess facial cosmetic surgery in a rejuvenation kind of style, so these were not appropriate. The Oral Health Impact Profile, providing valuable questions on pain, functional limitations or physical disability also has another scope.

CONCLUSION

This is the first case report to reflect CDT in edema following SRCCs. CDT even without compression is an effective treatment modality for minor external and suspected internal lymphedema and scar following oncologic surgery. As edemas are often accompanied by other symptoms, it is crucial to look for other suitable assessments to depict therapy's clinical efficacy.

The author concludes that there is no conflict of interest.

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THE LYMPHOSCINTIGRAPHIC STUDY OF THE DEEP LYMPHATIC CIRCULATION IN THE DIFFERENTIAL DIAGNOSIS OF OEDEMA OF THE LOWER LIMBS

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Key words: Two compartment lymphoscintigraphy, deep lymphatic system, leg oedema, leg lymphoedema.

ABSTRACT

a) Background
Lymphoscintigraphy is the main investigation for visualization of lymphatics, assessment of their function, diagnosis of lymphoedema. Till now there is no international agreed standard. Even if there are two peripheral lymphatic circulations (superficial and deep), with connections and the potentiality of compensations in case of lymphatic damage, commonly only the superficial lymphoscintigraphy is performed. This could lead to a wrong diagnosis of the origin of a leg oedema.

b) Aim of the study
Demonstration of the importance of performing lymphoscintigraphic study of both superficial and deep peripheral circulation (two compartment lymphoscintigraphy).

c) Material & Methods
A retrospective study was carried out on lymphoscintigraphic exams performed between 2013 and 2018, at Nuclear Medicine Unit of University Hospital of Udine (Italy), for diagnosis in case of established or suspected lymphoedema of lower limbs.
Lymphoscintigraphy of superficial and deep lymphatic systems has been performed on 142 patients.
Of these, 106 are females (75%) and 36 are males (25%), with average age of 52,7.
Eightyone patients have been diagnosed with lymphoedema, in congenital form for 37 and secondary for 33; 11 patients have been diagnosed with lipoedema, and 61 are affected by leg oedemas of unknown origin.
The lymphoscintigraphic assessment of the superficial circulation was followed, after few days, by the examination of the deep system.
In both studies, the images were taken immediately and after 60 and 120 minutes. They were evaluated semiquantitatively using a modified Transport Index score, with analysis of tracer kinetics, distribution pattern, and visualization of lymphnodes and lymphatic vessels. The resulting data were subjected to descriptive statistical analysis.

d) Results
In 31 cases the study was normal (TI <9) for both limbs.
In pathological cases, the involvement of the deep lymphatic system (mono- or bilateral, associated or not with similar disturbance of the superficial system) was found in 100 cases;
Involvement of the deep system alone was found in 29 cases (13 unilateral and 16 bilateral).
The exclusive involvement of the superficial system was found in 11 cases.
In the group of patients with leg oedema of unknown origin, lymphoedema was excluded in 18 cases, while in the remaining 43 cases there were all the combinations of superficial and deep lymphatic damage.

e) Conclusions
Our study demonstrates a high prevalence of deep lymphatics damage in case of leg lymphoedema. Furthermore, a two compartment lymphoscintigraphy allows the detection of isolated deep lymphatic impairment in case of leg oedemas of unknown origin, with early addressing to adequate care path.

INTRODUCTION

Oedemas of the lower limbs represent a disorder found at any age, even if they are more frequent in the elderly. They can be divided into two types, depending on the kind of lymphatics insufficiency: oedema depending on functional disturbance (venous oedema, with low level of proteins) and that due to mechanical impairment (oedema that is rich of proteins)[1].
In the first case it is a sign of an overload of a normal-functioning lymphatic system, with a lot of different possible causes; in the second case we face a well defined clinical entity: lymphoedema.

The differential diagnosis of these oedemas can become an important challenge, especially in mild and unilateral forms, since lymphedema can have at first stage a clinical expression similar to that of venous oedema. Lymphoscintigraphy, a minimally invasive diagnostic tool used for quantitative and qualitative study of lymphatic circulation, can be of great help

It allows the assessment of pathways of lymphatic drainage, quantification of lymphatic flow and differentiation of lymphedema from other oedematous conditions.[2, 3]

Its protocol is not standardized: main differences include the choice of radiotracer, use of dynamic and static acquisitions and acquisition times[4].

Another difference is if lymphoscintigraphy is performed for the study of the superficial circulation only, or if it includes also an examination of the deep lymphatic flow.

Actually the lymphatic circulation is organized in a superficial and a deep network, that at extremities run respectively above and under the muscular fascia.

The two lymphatic nets have some connections and one can compensate a malfunctioning of the other[5].

The study of both superficial and deep lymphatic nets is called two compartment lymphoscintigraphy[6]. Inclusion of the deep lymphatic vessels in the lymphoscintigraphic study has shown to increase diagnostic accuracy.[7]

In order to confirm the importance of the assessment of deep lymphatic circulation for lymphoedema detection, we present our casuistry, relating to established lymphedema conditions and leg oedemas of unknown origin.

MATERIAL AND METHODS

A retrospective analysis was performed on 142 two compartment lymphoscintigraphies of the lower limbs, carried out in the period 2013-2018 at Nuclear Medicine Institute of University Hospital of Udine (Italy).

Most of patients were addressed to the institute by an expert lymphologist. Other subjects were sent by specialists in Vascular Surgery or Infectious Diseases.

The following lines in italic are no "results" but rather description of the studied population and we move these lines in materials

We studied 142 patients (36 male and 106 female) with suspected or clinically diagnosed lymphoedema. The average age of the sample was 52.7 years (± 17.7).

The anamnestic and clinical evaluation prior to the examination made it possible to specify the diagnosis in 81 cases: 37 primary lymphoedema, 33 secondary forms and 11 lipoedemas with doubtful mechanical insufficiency of the lymphatics.

In 61 cases, two compartment lymphoscintigraphy was performed for diagnostic clarification in mono- or bilateral oedemas it would be interesting to give lateralisations of unilateral (right or left) edema of the lower limbs of unknown origin.

Referral pathology	1ary	2eary	lipoedema	unestablished	total
Total n	37	33	11	61	142
Age (mean+/-sd)					52.7+/-17.7
Male/female					36/106
Unilateral					
Right					
Left					
bilateral					

Proposed table for presentation of you populations

Technique of the lymphoscintigraphic examination

Lymphoscintigraphy of the lower limbs were performed in two phases: a study of the superficial circle followed, after a few days, by the study of the deep one.

For the assessment of the superficial circulation, an intradermal injection was performed at the 2nd intermetatarsal space of the distal portion of the dorsum of the foot; for the second study, the injection was performed subfascially, at the posterior portion of the sole of the feet.

Half milliliter of solution containing 99Tc-albumin nanocolloid (37 MBe) precise name of product, provider, how you reconstituted the tracer?
were injected in each site.

Static images were taken immediately after the administration of the tracer where? Why?
; other images which kind? Static ? WBS? Where?
were taken at one and two hours from the injection. Does it mean three sets of pictures?

Between the first and the second images set patients performed motor activity of the legs (walking) continued for 45 minutes. Not clear… Does it mean that there was no phase in resting conditions?

Data evaluation

A modified Transport Index was used to differentiate pathological and normal pictures.

Since the used protocol does not allow calculation of time to visualize the lymph nodes (T), only transport kinetics (K), distribution of the tracer (D), visualization of lymph nodes (N) and visualization of lymph vessels (V) were assessed. The pathology threshold has been reduced to 9.

The scores were assigned by a Nuclear Medicine medical doctor belonging to the Institute, who is expert in the execution and reporting of lymphoscintigraphies.

Show samples of normal and abnormal superficial and deep exam with corresponding analysis-values of your modified TI

Explain your gradation for K D N V

Statistical evaluation

A descriptive statistical analysis was performed, using Excel 2013 (Microsoft Corporation, Redmond, WA). Be more precise with regard to stat test used!

RESULTS

You give no stat analysis???
The following lines in italic are no "results" but rather description of the studied population and we move these lines in materials

We studied 142 patients (36 male and 106 female) with suspected or clinically diagnosed lymphoedema. The average age of the sample was 52.7 years (± 17.7).

The anamnestic and clinical evaluation prior to the examination made it possible to specify the diagnosis in 81 cases: 37 primary lymphoedema, 33 secondary forms and 11 lipoedemas with doubtful mechanical insufficiency of the lymphatics.

In 61 cases, two compartment lymphoscintigraphy was performed for diagnostic clarification in mono- or bilateral oedemas of the lower limbs of unknown origin.

	S+S	S+SD	N+N	SD+SD	D+D	D+S	N+SD	N+D	SD+D	N+S	total
Primary Lymphedema	0	4	4	7	4	0	4	5	8	1	37
Secondary Lymphedema	2	3	5	7	2	0	6	2	5	1	33
Lipedema	1	0	4	0	3	1	0	0	2	0	11
Suspected Lymphedema	2	3	18	5	7	5	6	6	5	4	61
Total	5	10	31	19	16	6	16	13	20	6	

Table n.1: Different patterns relating to damage to one and/or both legs (damage: S = superficial, D = deep, SD= superficial and deep; N = none). For example S+SD means damage of superficial lymphatic circulation on one side and damage of both superficial and deep circulation on the other side.

The results can be summarized as follows (see table 1):

- In 31 cases the studies of the superficial and deep lymphatics were normal (TI <9: see N+N cases in table 1) for both limbs.

- In 35 cases, studies of the superficial and deep lymphatics were normal (TI <9: see N+ S/D cases in table 1) for one of the two limbs.

- The superficial lymphatic exam was solely abnormal at the level of the two limbs in 5 cases (TI <9: see S+S cases in table 1) and at the level of the one limbs in 22 cases (TI <9: see S+ S/D cases in table 1)

- Etc one way to clarify this table 1 would be to divide it in four tables (with 1ary, 2ary, lipedeama and unknown origin) as follows

		Results of Superficial exam		
		normal	abnormal	total
Results of Deep exam	normal	(4x2)+10=18	(0x2)+5=5	(4x2)+15=23
	abnormal	(4x2)+13=21	(7x2)+16=30	(11X2)+29=51
	total	(8x2)+23=39	(7x2)+21=35	(15x2)+31=74

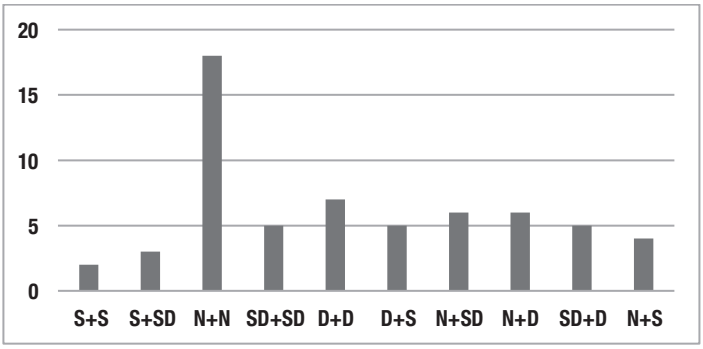
Table 1 a: results for patients (n=37) with 1ary LE

OK?
Do it for other groups!

And perform stat comparisons between groups?
In pathological cases, the involvement of the deep lymphatic system (mono- or bilateral, associated or not with similar disturbance of the superficial system) was found in 100 cases;

damage of the deep system alone was found in 29 cases (13 unilateral and 16 bilateral); the exclusive involvement of the superficial net was found in 11 cases (further specifications in table 1).

In the group of 61 patients in whom lymphoedema was suspected, according to the TI score, lymphoedema was excluded in 18 cases (30%), while in the remaining 43 cases there were all the combinations of damage (superficial / deep, mono- / bilateral), with greater frequency of exclusive bilateral involvement of the deep circulation)(see graph 1).



Graph n.1. Distribution of damage patterns in the group of patients with leg oedema of unknown origin.

DISCUSSION

Lymphoscintigraphy is the main investigation for visualization of lymphatics, assessment of their function, diagnosis of lymphoedema.

Till now there is no international agreed standard in relation to tracer, sites of injection, times of image acquisition.

Even if there are two peripheral lymphatic circulations (superficial and deep), with connections and with the possibility of compensations in case of lymphatic damage, commonly only the superficial lymphoscintigraphy is performed. This can lead to misdiagnosis of the origin of an oedema.

The possibility of studying superficial and deep lymphatic circulation of the leg was already presented by Bräutigam in the nineties[5,6], what he called two compartment lymphoscintigraphy, was developed in order to assess type and stage of leg oedemas. With this approach he could differentiate primary lymphoedema and early and advanced stages of postthrombotic syndromes.

Diagnosis of a leg oedema can be challenging at any age. Actually there are many factors to take in account: duration, mono- or bilaterality, location (proximal or distal), fovea and Stemmer signs, other known patient's pathologies (heart, renal or liver failure; chronic venous insufficiency, hypothyroidism and others). Important is the possibility of a detection and confirmation of a lymphoedema, but in early stages this pathology may have clinical characteristics similar to those of a venous oedema.

In our experience, lymphoscintigraphy of the lower limbs is performed for diagnostic confirmation and prognostic evaluation of a clinically diagnosed lymphedema, for an early diagnosis of mechanical insufficiency in lipoedema at the 2nd and 3rd stages, and for differential diagnosis in oedemas of unknown origin. For this last indication, we learned to perform the study early, particularly in case of mild, unilateral, distal oedema, which often proves to be a primary lymphedema at the 1st stage or at the second initial stage.

Since 2013 at the Nuclear Medicine Institute of University Hospital of Udine lymphoscintigraphy of the lower limbs involves the study of both the superficial and the deep circulation, in the belief that the data obtained allow better understanding of the lymphatic function and clinical correlations.

The high prevalence of deep lymphatic damage confirms the importance of the two compartment examination: of the 111 people with pathological lymphoscintigraphic exam, 100 cases showed a mono- or bilateral deep circulation impairment, associated or not with alteration of the superficial circle. This data agree with that recently published by Campisi, in which on a series of 248 two compartment lymphoscintigraphies the deep lymphatic vessels were damaged in the vast majority of patients (88% - 98%), with a pathological TI for either the deep subfascial vessels alone, or both the superficial and deep vessels[8].

In 29 cases we found a damage concerning only the deep lymphatics. This means that in more than a quarter of patients submitted to a study of the superficial circle alone, diagnosis of lymphatic impairment would be missed. This is in accordance with Villa's report: in his study deep vessel abnormalities were found in approximately 30% of the patients with normal superficial system[9].

A correlation has been demonstrated in the past between isolated damage of the deep lymphatics of the lower limbs and initial stages of postthrombotic syndrome; in advanced stages also the impairment of superficial lymphatics was found, as consequence of chronic functional overload of this net. A less frequent isolated alteration of the deep lymphatics was found also in case of primary lymphedema[5]. Our patient had neither history nor clinical features of a postthrombotic syndrome.

In the group of patients with oedema of unknown origin, the examination was diagnostic in 43 of 61 cases, confirming the importance of two compartment lymphoscintigraphy in the differential diagnosis of oedema, especially in the initial, clinically mild forms. Bräutigam was able to distinguish different lymphoscintigraphic patterns, finding: in case of idiopathic cyclic oedema, a marked increase in lymphatic flow in the two networks; increased flow in the superficial circle in case of phlebedema; in postthrombotic syndrome reduced flow in the deep network, while in the superficial it was normal or reduced depending on the duration of the condition; normal activity in obesity[6].

The possibility of distinguishing the origin of the swelling can allow early adoption of adequate therapeutic strategies (especially in the case of lymphoedema) or address to further investigations, in case of venous oedema of still unrecognized origin.

This possibility is also important in all those cases where an objective demonstration of a lymphoedema ensures specific benefits, provided by health systems.

The completion of lymphoscintigraphy with the study of the deep network can have implications for the surgical approach to lymphoedema. Derivative interventions between lymphatics and veins can be performed in two ways: connection of one superficial collector to one small venule in multiple sites (supermicrosurgery), or multiple lymphatic-venous anastomoses involving superficial and deep lymphatic vessels into a vein branch[10-12]. Knowledge of the functional state of the superficial and deep lymphatic vessels could guide the choice of one or the other surgical approach.

Our study presents some limitations. One of these is the modification of criteria for calculation of Transport Index, eliminating the T. It was due to our modality of study performance, that not allows the computation of this parameter.

Other authors have already used a modified TI score, without specification about the change, and with a cut off of 5[13].

The fact that lymphoscintigraphy is a non-standardized examination exposes to evident difficulties in applying score systems.

We could not make a correlation with the clinical data (extent of swelling, features of the skin) since, being a retrospective study, it was not possible to obtain all the necessary data. A study on this correlation will be carried out in further prospective studies.

We would appreciate that you will not forget our own work published by our group (Barbieux r et al) and that you will also comment your results with regard of our article…

OK?
You comment only surgical treatment… No comment for the physical treatment for deep lymphatic system?

CONCLUSIONS

Lymphoscintigraphy is an important tool for diagnosis of lymphoedema. Nevertheless, it is still lacking of a standardization. Moreover, due to many reasons, in most cases only an investigation of the superficial lymphatic circulation is performed. This can lead to misdiagnosis, especially in case of mild leg oedema of unknown origin.

Our study demonstrates that in a relevant (?) number of patients

One percentage here would be great!

the deep lymphatic circulation is damaged. This could be of interest not only for a diagnostic reason, but also for the choice of the best surgical approach to lymphedema.

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FREE FATTY ACID IN SECONDARY LYMPHEDEMA AND ITSCHANGE ATER HYPERTHERMIA THERAPY

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ABSTRACT

Free fatty acid is associated with inflammation and makes lymphedema worse. That is why it has been evaluated in lymphedema before and after hyperthermia treatment. It is increased as compared with the volunteers and decreased after the treatment.

INTRODUCTION

Lymphedema is closely associated with lipid because the fluid is deposited in the subcutaneous fatty tissue area and overweight people have more chance to develop post- operative lymphedema. Free fatty acid is associated with inflammation and makes the lymphedema worse. That is why it has been evaluated before and after the hyperthermia treatment. When we observe lymphedema patients they are becoming worse little by little after long clinical courses. The reason why is not known. However the author suspects a changed fat metabolism is involved in this clinical course. If we can stop this process, we may have a good clinical course in lymphedema and the patients will be happy about this.

OBJECTIVES

The penetration of oxygen into the cell is up to maximum 100 m μ and the size of the subcutaneous fat cells in lymphedema is sometimes bigger than the limit[1]. That is to say, the fat cells in the subcutaneous area in lymphedema suffer from asphyxia. A typical picture of fat cell necrosis is crown-like structure which has been shown in case of metabolic syndrome, lipiedema and also lymphedema[1]. Generally speaking oxygen content in lymph is smaller than blood and partial oxygen pressure in lymphedematous skin is lower than uninvolved one[2]. In lymphedema fluid is deposited in the subcutaneous area as MRI picture shows. All these suggest lymphedema is associated with DAMP(damage associated molecular pattern).

MATERIAL AND METHOD

Serum free fatty acid, total cholesterol and triglyceride are evaluated in secondary lymphedema patients and in the volunteers(number of examined persons is listed in the Table 1). The volunteers are those who are between 40 and 89 years old and have been preoperatively examined for the purpose of operation of benign tumors. Free fatty acid has also been counted by chemical method(standard value: 150-600 mEq//L) in the secondary lymphedema patients before(42 cases) and after hyperthermia treatment(11 cases)[3](Fig. 1). All are females and those who are diabetic, cardiac, hyperlipidemic, hyperthyroidic and pheochromocytomic patients or have BMI bigger than 25 have been excluded from the data. Sera are taken from the patients in the early morning after starvation since the previous night's regular and caffeine-free dinner.The number of investigated patients and volunteers are mentioned in each examination.

		mean age	increased	standard	decreased	total
I.	FFA, patient	70	19(48%)	23(5%)	0	42
II.	FFA, volunteer	70	1(13%)	7(87%)	8	16
III.	TC, patient	70	11(33%)	22(67%)	0	33
IV.	TC, volunteer	82	6(20%)	20(67%)	4(13%)	30
V.	TG, patient	70	22(18%)	99(82%)	0	121
VI.	TG, volunteer	84	4(21%)	17(89%)	0	21

FFA:free fatty acid, TC:total cholesterol, TG:triglyceride
I vds II:p-value <0.02, III vds IV:p-value>0.05, V vds VI:p-value>0.5

Table 1. Number of Cases in Free fatty Acid, Total Cholesterol and Triglyceride in Blood Evaluated in Secondary Lymphedema and in Senile Volunteers.

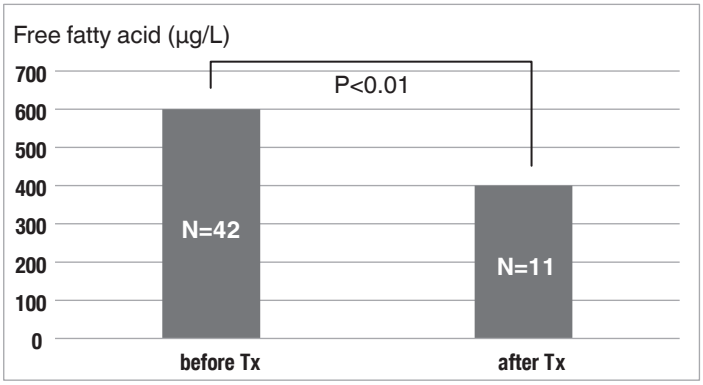

Fig. 1 - Free fatty acid in secondary lymphedema(42 cases) and its change after hyperthermia treatment (11 cases). It has been decreased.

RESULT

Free fatty acid is higher in lymphedema patients than in the volunteers($p<0.02$). However total cholesterol is not higher in the lymphedema patients than in volunteers($p>0.05$). Neither is triglyceride in the lymphedema patients than in the volunteers($p>0.5$)(Table 1). Free fatty acid is decreased in lymphedema patients after the treatment. Forty two patients.are compared with 11 cases after the treatment[3],(Fig. 1).

DISCUSSION

Usually 5% of all blood fatty acid is free. It is produced from the fatty tissue by hydrolysis of triglyceride. Free fatty acid stimulates macrophage resulting in inflammation via

ER stress,UPR(unfolded protein response) and angiopoietin like protein 2(alarm signal). Continous oxygen deficit of fat cell due to partly enlarged adipocyte in lymphedema results in production of DAMP(damage associated molecular pattern) which results in stimulation of TLR 4(Toll like receptor 4) as well as increase of TNFα. Thus a kind vicious cycle is established and a tissue remodeling advances. In this condition adiponectin becomes decreased and the tissue changes advance(Fig. 2,3)..The author has al- ready demonstrated oral EPA combined with hyperthermia treatment for secondary lymphedema improves clinical treatment effect(World Congress of Lymphology, Rome, 2013). EPA lowers TLR 4 signals and decreases TGF-beta 1(Fig.3). End product of EPA, resorbin E is a kind of anti-inflammatory substance. In future the mechanism how the inflammation of lymphedema has been decreased after the hyperthermia treatment must be investigated by means of M1 macrophage, beige brown fat cell, crown like structure and, heat schock protein,etc..

Fatty tissue→hydrolysis of triglyceride→ FFA→
→ER stress(unfoldeded protein response)
→angiopoietin like protein 2(alarm signal)
→stimulated macrophage producing TNFα
→homeostatic inflammation
→tissue remodeling(fibrosis of tissue, increase of TGF β 1)
→worsening of lymphedema

Fig. 2. The mechanism how lymphedema gets worse by FFA.

partly obase fat cell →oxygen deficit→MCP-1* from fat cell→
M1 macrophage ↑ →TNFα →inflammatory adipocain ↑ , adipolysis ↑
ǂ ↑
EPA FFA(ligand of TLR4)**
***MCP-1:macrophage inducible protein-1**
****TLR4:Toll like receptor 4**
ǂ:inhibit
↑ increase

Fig. 3 What happens in the lymphedema tissue in relation with FFA

SUMMARY AND CONCLUSION

Free Fatty acid is evaluated in secondary lymphedema before and after the hyperthermia treatment. Elevated free fatty acid has been decreased after the treatment.

ACKNNOWLEDGEMENT

This work has been presented in World Congress of Lymphology, Barcelona, 2017.

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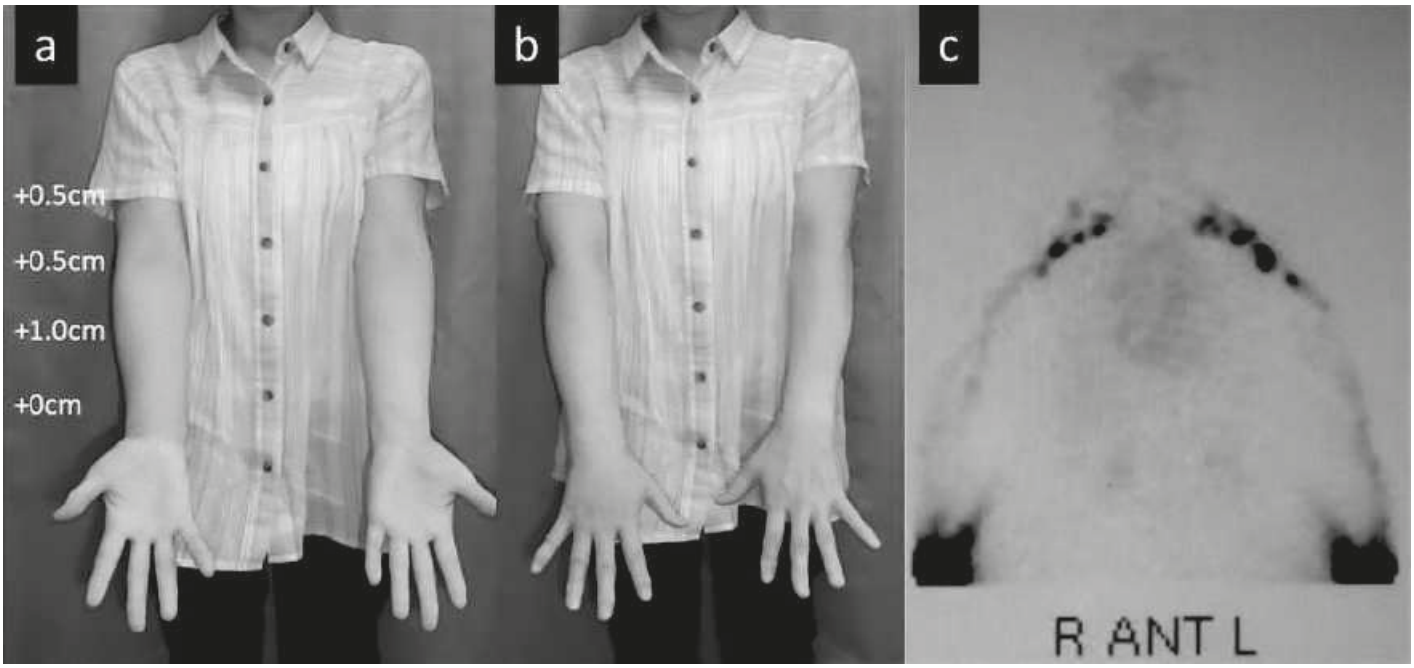
BOURGEOIS PER EJLRP TOO MUCH IS TOO MUCH ISL 2021



«Too much is too much»: Mood swings about «superiority» and misplaced comparisons during one international meeting…

On 20 and 21 September, I attended various sessions of the International Lymphology Congress in Athens.

First annoyance: the comparison between an MRI lymphangio and a lymphoscintigraphy, the first being said to show things not seen by the second. I was aware of these images already presented in other meetings, but when I look at this lymphoscintigraphy, I still don't think it's normal and I'm still convinced that, more contrasted, it would have shown the same anomalies as the MRI.

Second annoyance: our japanese colleagues are here but cited two of their previous work (which i will repeat below) as « » evidence of the superiority of ICG lymphofluoroscopy over scintigraphicimaging. During their publications,
I had already ticked but there it is too much («errare humanum est sed perseverare diabolicum est»). And now I reply:

Read the following article:
Indocyanine green (ICG) lymphography is superior (???) to lymphoscintigraphy for diagnostic imaging of early lymphedema of the upper limbs.
Mihara M, Hara H, Araki J, Kikuchi K, Narushima M, Yamamoto T, Iida T, Yoshimatsu H, Murai N, Mitsui K, Okitsu T, Koshima I.PLoS One. 2012;7(6):e38182. doi: 10.1371/journal.pone.0038182. Epub 2012 Jun 4.

1st remark:
I quote «With the patient in the supine position, a radioisotope, 99mTc, was subcutaneously injected into the bilateral second interdigits (0.2 ml each, 0.4 ml in total, 80 MBq).»

1st review:
a) what is this lymphoscintigraphy where the radioisotope alone is injected, pertechnetate, 99mTcO4, which normally has to be coupled to a carrier molecule (the peer-review « » was a «pear-review»)?
b) Their image, which I show below, shows the presence of free technetium by the fact that we visualize the circulating activity of the heart.

2nd remark:
I quote «Images of the whole affected arms were acquired using the scinticamera after 30, 60, 90, and 120 minutes. After subcutaneous injection, the injected regions were mildly massaged, but...(see next remark)

2nd review:
The repetition of the images at these intervals means that these patients are at rest (otherwise without exercise, see 3rd remark) and their massage is insufficient only to charge the lymphatic vessels

3rd review:
the authors of these lymphoscintigraphs make a subcutaneous injection of their radioisotope, but on the other hand, for ICG

lymphofluoroscopy, they inject Indocyanine Green «intracutaneously». The point is important because an intradermal treatment gives rise to a much greater clearance and lymphatic extraction than a subcutaneous one.

3rd remark:
I quote «... but no exercise load was applied to the four limbs»

4th review (basic):
4 members? a patient has only two arms that I know of... It's not serious.

4th remark:
I quote «Observation of dermal backflow of the radioisotope was taken as an indication of lymphedema»

5th review:
the authors choose their lymphoscintigraphic criterion identical to that which they choose for lympho-fluoroscopy but the possibility of having a dermal reflux on lymphoscintigraphy is diminished by their insufficient scintigraphic methodology and I am not sure that more contrasted the image proposed as an example «normal» in their article (and presented at the end of this letter) would not have revealed a reflux at the level of the right forearm...

5th remark;
I quote from the discussion «lymphoscintigraphy has disadvantages of radiation exposure, high invasiveness, high costs, and low resolution»

Indocyanine green lymphography is superior to lymphoscintigraphy in imaging diagnosis of secondary lymphedema of the lower limbs.
Mihara M, Hara H, Narushima M, Todokoro T, Iida T, Ohtsu H, Murai N, Koshima I.J Vasc Surg Venous Lymphat Disord. 2013 Apr;1(2):194-201. doi: 10.1016/j.jvsv.2012.07.011. Epub 2013 Feb 15.

And then the third:
Multilymphosome injection indocyanine green lymphography can detect more lymphatic vessels than lymphoscintigraphy in lymphedematous limbs.
Hara H, Mihara M.J Plast Reconstr Aesthet Surg. 2020 Jun;73(6):1025-1030. doi: 10.1016/j.bjps.2020.01.021. Epub 2020 Jan 21.

In this last article I quote "ICG lymphography was performed by injecting ICG in three lymphosomes per limb: dorsum of foot (saphenous lymphatics), the proximal side of the lateral condyle (lateral calf lymphatics), and the lateral side of the superior edge of the knee (lateral thigh lymphatics). We observed the presence or absence of a linear pattern at each injection site with a near-infrared camera. Lymphoscintigraphy was performed by injecting **an isotope" -see 1rst review after a)- " in the first web space**, conventionally. Whole body scintigrams were taken 60 min after injection.»

Let's be serious. Did you not expect that 3 injections per foot would lead to more visualization of lymphatic vessels than a single one... In addition, planar scintigraphic imaging alone may be unable to identify lymphatic vessels under dermal reflux when a dynamic lymphoscintigraphic approach would have allowed it.

COMMENTS:

- The problem of radiation is a problem perfectly mastered by specialists in Nuclear Medicine who unfortunately can do nothing against the «radiationphobia» of some.
- And ICG users seem to be blind to the literature's references to potential ICG toxicity on lymphatics...
- The invasiveness of lymphoscintigraphy; how are injections of ICG less invasive than those of radiocolloids?
- Finally, the argument «high costs» for lymphoscintigraphy must be specified with supporting figures and the nuclear medicine techniques that apply to all organs of the human body must be compared with the limited indications of ICG lympho-fluoroscopy (whose cameras are comparatively expansive).

The same with their second article for the lower limbs with the same methodological and analytical remarks about the images presented that are not sufficiently contrasted.

IN CONCLUSIONS:

In short, never draw conclusions from certain articles or from the comparison of certain images.

«You can be an apple merchant and prefer them to pears but present rotten pears as an argument, it borders on dishonesty (intellectual for a scientist).»

Computer scientists have an applicable acronym here. As follows: «GIGO» for «Garbage In, Garbage Out».

To certain colleagues specialized in Nuclear Medicine who carry-carried out these lymphoscintigraphies: «If you do these lymphoscintigrams, do them as well as you can... or don't do them».

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AUTOLOGOUS PERIPHERAL BLOOD MONONUCLEAR CELLS FOR THE TREATMENT LOWER EXTREMITY LYMPHEDEMA: 4 CASE REPORT

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ABSTRACT

INTRODUCTION *Lymphedema is the clinical manifestation of damaged lymphatic transport due to impaired lymphatic drainage which cause proteins and lipids build up in the interstitial space, tissue progressive architectural changes such as adipose tissue deposition and fibrosis. These changes are also strongly associated with inflammation which cause resistance to current therapies making lymphedema both under-recognized and under-documented clinical condition that still lacks a cure. Lower extremity lymphedema has a strong negative impact on quality of life. Therefore, innovative treatments are needed to restore the functionality and integrity of the lymphatic vessels damaged by this pathology. Recently, cell therapy has emerged as a new therapy in the treatment of lymphedema because a therapy able to supporting the restoration of the lymphatic vascular system from the capillary to the collector level is still needed. Recently, Autologous Peripheral Blood Mononuclear Cell (PBMNC), consisting of autologous monocytes/macrophages and lymphocyte, showed to be able to induce therapeutic angiogenesis in the treatment of critical limb ischemia and more generally in the healing of chronic non-healing wound. These cell therapy used to treat vascular diseases may also be applicable in the treatment of lymphatic diseases. Lymphangiogenesis, as angiogenesis, occur in response to tissue damage or in the presence of pathology like cancer, and in this environment, an increase in the number of activated macrophages has been observed. Based on this rationale we decided to treat patients affected by primary lymphedema of lower limbs, which not responding to standard therapy, with implantation of autologous PBMNC in the lymphedema tissue.*

MATHERIAL AND METHODS *We enrolled 4 patients (3 men and 1women), male aged 36, 74 ,75 years, and female aged 54 years, with 3rd clinical stage lymphedema of the lower limbs, in particular with primary lymphedema in 2 cases, one post-surgical case and 1 post-lymphangitic case.*

All patients underwent metric evaluation of the circumferences above-below the knee, calf, ankle and on the back of the foot, and an instrumental evaluation with arterial and venous doppler ultrasound of the lower limbs, longitudinal ultrasound measurement of the medial portion of the knee, the calf, the internal and back foot malleolus, before and after the three PBMNC implantation steps. Diagnostic examinations were performed including high-resolution ultrasound of soft tissues in all patients (both at the beginning and at the end of treatment). After each treatment session, each patient was subjected to a zinc-oxide and coumarin multilayer bandage. Oral therapy with 100 mg of Melilotus (containing 20% coumarin equal to 20 mg), 300 mg of Rutin and 100 mg of Bromelin, one tablet /day was also prescribed for all patients. Patients were assessed at baseline (T0), after one months (T1), and after two months from the implant (T2), after five months (T3) for the following parameters: pitting, measurement of circumferences of the limbs, measurement of ultrasound thicknesses of the superficial tissues in the affected limbs, VAS and assessment of the patient's well-being through the SF12 questionnaire. To determine the effectiveness of this treatment, final endpoint to evaluate improvements were decrease in the limb circumference, volume reduction in the lymphoscintigraphic transport index (TI), increase in the lymphatic vessel count. Moreover the quality of life of patients were evaluated. Lymphoscintigraphy was performed before end after the treatment. Patients with acute relapsing lymphangitis were included, while secondary lymphedema to cancer intervention less than 10 years were excluded. All patients signed a statement to consent the publication of their data.

Follow-up. The patients were followed for 4 months and for the whole period subjected to a zinc oxide and coumarin multilayer bandage with weekly replacement after decongestive treatment with Combined Decongestive Physic Lymphatic Treatment (Co.De.Phy.L).

***RESULTS** In all 4 treated patients there was a significant clinical improvement with disappearance or significant reduction in pain and / or heaviness already in the days immediately following the first implantation of mononuclear cells such as not to require any analgesic treatment. In addition, none of the patients evaluated had side effects or adverse reactions such as lymphangitis or septic lesions at the inoculation site after treatment. Used on patients with elephantiasis from lymphedema of the lower limbs, the implantation of mononuclear cells (PBMNC) since the first cycle, has given decongestive results that exceeded expectations, with a rapid reduction in the volume of the limbs of 60-90%, in subsequent cycles, in all four patients, with rapid healing of trophic lesions and a significant improvement in the patient's quality of life. Measurement of limb volume reduction is the most common approach to quantify the extent of lymphedema and evaluate therapeutic success. After the first implant (3-4 weeks after T1) 24.5%, on the second visit (3-4 weeks after the second T2 implant) 18.5%, and after five months after the start of therapy (T3) 15.3 %. All these volume reductions were significant compared to the baseline mean volume ($p < 0.05$).*

Lymphoscintigraphy was performed before end after the treatment.

Immediately after the first implantation of PBMC cells, we highlighted a significant reduction in the circumferences of the limb with a clear clinical improvement perceived by the patient as a "sense of lightness with restoration of sensitivity on the whole limb".

***DISCUSSION** The PBMNC implantation has proved, in the reported cases, effective and certainly well tolerated, without side effects; from a clinical point of view, a clear clinical and functional improvement was noted in all patients treated, regardless of the cause of the lymphedema; in patients with primary lymphedema there was a significant clinical improvement with recovery of normal functional activities. At scintigraphy , there is the visualization of the popliteal and inguinal lymph node stations and by hyper fixation of the tracer along the ipsilateral iliac axis not appreciable in the study carried out before treatment. In patients with secondary lymphedema a clear clinical improvement was not correlated to a significant response to the lymphoscintigraphic control, even if minimal but important signs of recovery suggest the possibility of obtaining, even in these patients in whom the district lymphatic patrimony has been surgically depleted , a neo-lymphogenesis by increasing or doubling the number of treatment cycles. Further controlled clinical trials are needed to demonstrate the efficacy of autologous PBMC implantation in the treatment of primary and secondary lymphedema.*

Keywords: Lymphoscintigraphy; Peripheral blood mononuclear cells; lower extremity lymphedema; cell therapy

INTRODUCTION

Lymphedema is the clinical manifestation of damaged lymphatic transport due to impaired lymphatic drainage which cause proteins and lipids build up in the interstitial space, tissue progressive architectural changes such as adipose tissue deposition and fibrosis. These changes are also strongly associated with inflammation which cause resistance to current therapies [1,2] making lymphedema both under-recognized and under-documented clinical condition that still lacks a cure[3]. Ninety percent of primary lymphedema occurs in the lower extremities and is bilateral in 50% of cases and, although ~90% of patients with lymphedema can be diagnosed by history and physical examination, confirmation requires lymphoscintigraphy which is useful to provide a quantitative measure of the results of an intervention[4]. Moreover, lower extremity lymphedema has a strong negative impact on quality of life[5]. Thus, innovative treatments are needed to restore the functionality and integrity of the lymphatic vessels damaged in this pathology. Recently, cell therapy has emerged as a novel therapy in lymphedema treatment [6-8] because a therapy able to supporting the restoration of the lymphatic vasculature from the capillary to the collector level is still needed. Recently, Autologous Peripheral Blood Mononuclear Cell (PBMNC), consisting of autologous monocytes/macrophages and lymphocyte, showed to be able to induce therapeutic angiogenesis in the treatment of critical limb ischemia and more generally in the healing of chronic non-healing wound [9-14].

Main mechanism of action of PB-MNCs is angiogenesis induction in order to promote collateral vessel formation through paracrine activities of growth factors, cytokines, messenger molecule and exosomes [15–17]. Moreover both monocyte/macrophages and lymphocytes Treg populations, play a key role in tissue regeneration in not-healing trophic lesions [18,19], also through polarization from macrophages inflammatory M1 phenotype to the anti-inflammatory regenerative phenotype M2 [20–22].

Great progress has been achieved in developing cell therapy to enhance vascular regeneration as showed in a recent meta-analyses on no option critical limb patients [23]. Comparing autologous cell therapy, PBMNCs showed to be only cell therapy able to statistically reduce limb amputation in critical limb ischemia patient non feasible for revascularization. These cell therapy used to treat vascular disease may also be applicable in the treatment of lymphatic diseases. Lymphangiogenesis, as angiogenesis, occur in response to tissue damage or in the presence of pathology like cancer, and in this environment, macrophages are activated

increased number were observed [24]. Both blood vascular microcirculation and lymphatic circulation are the conduits for the entry and the exit for monocyte-derived macrophages in almost every tissue. PBMNCs therapy is mainly based on the mechanism of action of monocytes, which differentiate in macrophages when from the blood enter in the tissue, and lymphocytes. The role of macrophages as key players in both angiogenesis and lymphangiogenesis is well known [24, 25]. Macrophages support lymphangiogenesis by two different mechanism of action, either by transdifferentiating and directly incorporating into the endothelial layer or by stimulating division of pre-existent local lymphatic endothelial cells [23]. These findings confirm the plasticity of macrophages, which are already known to transform from naive monocytes into VEGF-C–producing cells, as extensively reported in the review of Corliss et al [26], as VEGF-C is a key mediator of lymphangiogenesis C [27]. Macrophages represent the cellular link which spatially and temporally connects angiogenesis with lymphangiogenesis, in both physiological growth and in pathological adaptations [26]. It has been observed that T regulatory (Treg) lymphocytes cells limit the pathological changes in lymphedema tissue [28]. Accordingly, the depletion of Treg cells exacerbates edema and fibrosis and an increased immune cells infiltration [28]. In contrast, T regulatory cells expansion significantly reduced lymphedema by decreasing the tissue inflammation [28]. Based on this rationale we decided to treat patients affected by primary lower extremity lymphedema not responder to standard therapy with implantation of autologous PBMNC in the lymphedema tissue.

MATHERIAL AND METHODS

Study subjects.

We enrolled 4 patients (3 men and 1women), aged male 36, 72 ,75 years, and 55 years the woman, with 3rd clinical stage lymphedema of the lower limbs, in particular with primary lymphedema in 2 cases, one post-surgical case and 1 post-lymphangitic case.

In particular:

1) P.M.R. - 54 year-old woman
   - Primary lymphedema for 40 years
   - Elephantiasis for lower limbs for 20 years
   - Various press therapy and lymph drainage sessions
   - Repeated episodes of lymphangitis

2) N.M. - 75 year-old man
   - Radical prostatectomy
   - Increase in limb volume after about 30 days from surgery
   - Various press therapy and lymph drainage sessions

3) S.G. - 36 year-old man
   - About 10 years ago repeated episodes of inguinal lymphadenitis and lymphangitis
   - Within a year, a progressive increase in the volume of the lower right limb
   - 7 years ago lymphatic surgery failure (venous lymphatic anastomosis)
   - Various press therapy and lymph drainage sessions

4) S.M.B. - 74 year-old man
   - Primary lymphedema for 40 years
   - Elephantiasis for lower limbs for 30 years
   - 10 years ago lymphatic surgery failure (venous lymphatic anastomosis)
   - Various press therapy and lymph drainage sessions
   - Repeated episodes of lymphangitis

All patients underwent metric evaluation of the circumferences above-below the knee, calf, ankle and on the back of the foot, and an instrumental evaluation with arterial and venous doppler ultrasound of the lower limbs, longitudinal ultrasound measurement of the medial portion of the knee, the calf, the internal and back foot malleolus, before and after the three PBMNC implantation steps.

Diagnostic examinations were performed including high-resolution ultrasound of soft tissues in all patients (both at the beginning and at the end of treatment).

After each treatment session, each patient was subjected to a zinc-oxide and coumarin multilayer bandage.

In accordance with current therapeutic guidelines [29], oral therapy with 100 mg of Melilotus (containing 20% coumarin equal to 20 mg), 300 mg of Rutin and 100 mg of Bromelin, one tablet /day was also prescribed for all patients.

Patients were assessed at baseline (T0), after one months (T1), and after two months from the implant (T2), after five months (T3) for the following parameters: pitting, measurement of circumferences of the limbs, measurement of ultrasound thicknesses of the superficial tissues in the affected limbs, VAS and assessment of the patient's well-being through the SF12 questionnaire.

The volume measurements were made according to the disk model of Kuhnke[29]

The volume was calculated using the following formula:

$V= (C12+C22+\ldots+Cn2)/\pi$.

The percentage reduction in arm volume at each point of measurement was calculated via the formula:

ΔV%=[(pretreatment arm volume - post-treatment arm volume)/ pretreatment arm volume] x100

Statistical analyses were performed with the Student t-tests. Differences were accepted as significant when $p<0.05$. All patients gave informed consent before inclusion.

To determine the efficacy of this treatment, final endpoint to evaluate improvements were decrease in the limb circumference, volume reduction in the lymphoscintigraphic transport index (TI), increase in the lymphatic vessel count. Moreover the patients' quality of life were evaluated. Lymphoscintigraphy was performed before end after the treatment.

The methodology involves the inoculation of autologous mononuclear cells from peripheral blood with monthly sessions (maximum 30-40 days) for a total of three consecutive implants.

Inclusion and exclusion criteria

Patients with acute relapsing lymphangitis were included, while secondary lymphedema to cancer intervention less than 10 years were excluded.

All patients signed a statement to consent the publication of their data.

Autologous PB-MNC implantation.

Autologous PB-MNCs were produced by Pall Celeris / MonoCells Kit (Pall Medical, Athena Biomedical Innovation) through selective filtration system point-of-care device with the intended for use intra-operatively or at the point-of-care, for the rapid preparation of TNC concentrate from 120 mL of anticoagulated blood, for use in human cell therapy applications. The PBMNCs cell product obtained has been extensively characterized in terms of number of cells obtained, composition, recovery and FACS cell population analysis[31]. All the procedures were performed in operatory room with anaesthesiologic support (propofol and/or peripheral block). Briefly, 120 ml of anticoagulated peripheral blood were collected through a peripheral venous access and PBMNC were concentrated according to the manufacturer's instructions. The selective gravity filtration was allowed to proceed until all the blood was filtered; filtration last about 10 minutes. During filtration, MNCs were captured in the filter while plasma, platelets (PLTs) and red blood cells (RBCs) were discarded. The enriched MNCs were harvested by 10 mL of sterile saline backflush and collected in a cells recovery bag. After cell preparations, the patients were lightly sedated and 10 ml of autologous fresh PBMNCs concentrate were immediately implanted in lymphedemas tissue in 0.2–0.3cc in boluses, at intervals of 1–2 cm and to a mean depth of 1.5–2 cm, using a 21G needle.

The implants were performed in the back of the foot, ankles, popliteal cavity, inguinal region (locations of the main lymph node stations) and along the course of the main lymphatic collectors on the leg and thigh.

The PBMNCs implant procedure was repeated three times, at intervals of 30-45 days from each other. MNCs were concentrated 4.2 folds of the baseline, with an average of 1.06 +/- 0.28 $X10^8$ total PBMNCs injected (0,16 +/- 0.04 $X10^8$ Monocytes,0.9+/- 0.2 $X10^8$ lymphocytes). No mobilization was performed to increase the percentage of CD34+ stem cells, which were present in the concentrated achieving 5.6-fold enrichment after Pall Celeris filtration (from 0.32% in whole blood to 1.79% in Pall Celeris filtered PBMNC), with a mean count of 1.37X 10^6.

Follow-up.

The patients were followed for 4 months and for the whole period subjected to a zinc oxide and coumarin multilayer bandage with weekly replacement after decongestive treatment with Combined Decongestive Physic Lymphatic Treatment (Co.De.Phy.L) . The Co.De.Phy.L. treatment is an original idea of the Phlebo-Lymphology Clinic of the Complex Operative Unit of Emergency Surgery at the University Hospital of Palermo that arises from the experience done in some case of phlebo-lymphatic edema caused by post-thrombotic syndrome made by subjecting the patient to some decongestive sessions using a device capable of sequentially exploiting the effects of the Lipolaser, RF and Ultrasound[32].

The bilateral limbs' circumference and patients' weight were recorded monthly from the beginning. At the end of the 4-month follow-up period, lymphoscintigraphy was performed and the QOL questionnaire was given to each patient.

RESULTS

In all 4 treated patients there was a significant clinical improvement with disappearance or significant reduction in pain and / or heaviness already in the days immediately following the first implantation of mononuclear cells such as not to require any analgesic treatment.

In addition, none of the patients evaluated had side effects or adverse reactions such as lymphangitis or septic lesions at the inoculation site after treatment.

Used on patients with elephantiasis from lymphedema of the lower limbs, the implantation of mononuclear cells (PBMNC) since the first cycle, has given decongestive results that exceeded expectations, with a rapid reduction in the volume of the limbs of 60-90%, in subsequent cycles, in all four patients, with rapid healing of trophic lesions and a significant improvement in the

patient's quality of life, as evidenced by the VAS scale with an unexpected clinical improvement immediately after the first session, which was maintained in the subsequent sessions. (Fig.1) These data were confirmed by the completion of the SF12 questionnaire, which showed a progressive improvement in the quality of life.

Measurement of limb volume reduction is the most common approach to quantify the extent of lymphedema and evaluate therapeutic success. After the first implant (3-4 weeks after T1) 24.5%, on the second visit (3-4 weeks after the second T2 implant) 18.5%, and after five months after the start of therapy (T3) 15.3 %.

All these volume reductions were significant compared to the baseline mean volume ($p < 0.05$). (Table 1)

Lymphoscintigraphy was performed before end after the treatment.

- FIRST CASE (PMR): In the first case, patient suffering from elephantiasis of the lower limbs from primary bilateral lymphedema underwent re-evaluation of the superficial lymphatic drainage, through the execution of lymphoscintigraphy, after having performed three cycles of PBMNC implantation in the most affected sites of the left limb , which was the one clinically more severe.

From the comparison with the "baseline" scintigraphy study, some aspects emerged which suggest effectiveness of the treatment in patients with primary lymphedema.

From the very first images relating to the "dynamic" phase of the post-treatment scintigraphy study, it was possible to document a rapid ascent of the radiocolloid from the administration site of the left limb, unlike what was observed in the previous treatment which no progression of the radiopharmaceutical was appreciated from the injection site. The subsequent detection, carried out about twenty minutes after administration, keeping the patient in a supine position, also allowed us to highlight important changes in the distribution of the tracer to the left limb, which also appeared clinically more affected by the pathological process.

The presence of non -homogeneous accumulation of the colloid not only in the lateral side of the foot, as previously noted, but also in the distal third of the leg and in particular along the lateral side, which was the site of implantation of PBMNC cells, was highlighted early.

Further substantial variations emerged in the late phase of the study which involves the acquisition of images relating to lymphatic drainage 120 minutes after the administration of the radiopharmaceutical substance and after having invited the patient to walk. It was possible to document the appearance of more intense accumulation of the radiocompound at the level of the left inguinal lymph node station, initially only blurred, as well as further tracer hyper fixations, pertaining to the lymph node, in the popliteal cavity and along the ipsilateral iliac axis not appreciable in the previous measurement.

The re-evaluation of the superficial lymphatic drainage by means of a scintigraphic study was also performed in patients with secondary lymphedema of the lower limbs after the three treatment cycles.

In one of these patients, despite the objective improvement of the lymphedema, the expected results were not obtained from the scintigraphic point of view.

- SECOND CASE (NM): Patient with a history of operated prostate cancer; subjected to implantation of PBMNC in the most affected areas of the left limb.

In this patient, post-treatment lymphoscintigraphy confirmed in the left limb, in the dynamic phase, the failure of the radiocolloid to rise from the injection site.

Also in the subsequent acquisitions made at 20, 60 and 120 minutes (after stimulus) from the administration of the radiopharmaceutical no significant changes were observed compared to the “baseline” study; the presence of nuanced colloidal stagnation in both feet and in the distal third of the leg bilaterally was confirmed in the later images of the study and after stimulation, unchanged respect to what previously emerged. Furthermore, in all the surveys carried out, the lymphatic passages or clear areas of accumulation of the radiopharmaceutical referable to lymph nodes were not detected bilaterally.

- THIRD CASE (SG): Patient with a history of primary scrotal lymphedema and repeated episodes of bilateral inguino-crural lymphangio-adenitis; about 7 years ago underwent to lymphatic-venous anastomosis surgery with persistence of the lymphedema.

Subjected to implantation of PBMNC in the most affected areas of the right limb.

In the dynamic phase of the post-treatment scintigraphic study, the absence of the colloid migration from the inoculum site is confirmed in the right limb. 20 minutes after administration, a faint stagnation of the radiopharmaceutical appears on the back of the right foot, not noticeable in the previous study; the finding in the later phases of the study tends to extend to a slightly greater extent than documented in the "basal" study with progressive and further involvement of the middle and distal third of the leg, especially on the medial side, site of PBMNC implant.

- FOURTH CASE (SMB) – Patient with a history of primary lymphedema for 40 years; elephantiasis of the lower limbs for 30 years; 10 years ago underwent to lymphatic surgery (lymphatic-venous anastomosis); various sessions of press therapy and lymphatic drainage; repeated episodes of lymphangitis.

Pre-treatment basal lymphoscintigraphy confirmed in the left limb, in the dynamic phase, the failure of the radiocolloid to rise from the injection site. Even in the subsequent acquisitions made at 20, 60 and 120 minutes (after stimulation) from the administration of the radiopharmaceutical, no sign of progression of the tracer was recorded. Only in the later images of the study and after stimulation, was detected the presence of nuanced colloidal stagnation in the left foot and bilaterally in the distal third of the leg.

Immediately after the first implantation of PBMC cells, we highlighted a significant reduction in the circumferences of the limb with a clear clinical improvement perceived by the patient as a "sense of lightness with restoration of sensitivity on the whole limb".

DISCUSSION

Despite the few preliminary cases dealt with, the observed data suggest that

1) The PBMNC implantation has proved, in the reported cases, effective and certainly well tolerated, without side effects, even by patients suffering from lymphedema of the lower limbs easily susceptible to infections and lymphangitic reactions.

2) From a clinical point of view, a clear clinical and functional improvement was noted in all patients treated, regardless of the cause of the lymphedema. A rapid and progressive decongestion with reduction in volumes was noted in all patients. All patients, both those with primary and secondary lymphedema, reported in the period following the first session, a sense of "lightness" and a fluency in limb movements never experienced with the current pathology.

3) In patients with primary lymphedema there was a significant clinical improvement with recovery of normal functional activities. The significant clinical improvement found correspondence with the lymphoscintigraphic data characterized, at five months, by the visualization of the popliteal and inguinal lymph node stations and by hyper fixation of the tracer along the ipsilateral iliac axis not appreciable in the study carried out before treatment.

4) In patients with secondary lymphedema a clear clinical improvement was not correlated to a significant response to the lymphoscintigraphic control, even if minimal but important signs of recovery suggest the possibility of obtaining, even in these patients in whom the district lymphatic patrimony has been surgically depleted , a neo-lymphogenesis by increasing or doubling the number of treatment cycles.

Further controlled clinical trials are needed to demonstrate the efficacy of autologous PBMC implantation in the treatment of primary and secondary lymphoedema. PBMNC are safe and effective in critical limb ischemia treatment, diabetic foot, and non-healing chronic wound. Moreover, the treatment with PBMNC is autologous, non- invasive for the patients, repeatable, easy and fast to perform in OR. We believe that we are at the very beginning of this exciting adventure but the good clinical data response obtained in these critical patients, together the lymphoscintigraphic measurement, suggest that we could be on the right path.

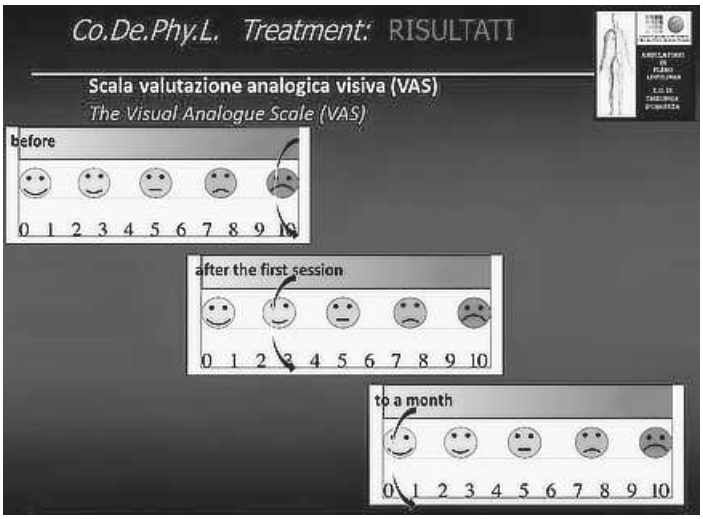
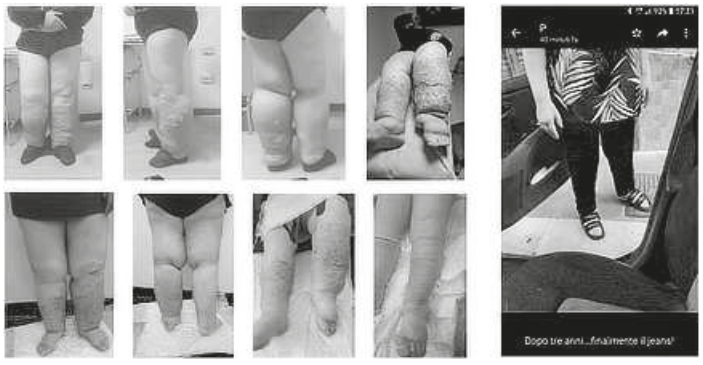


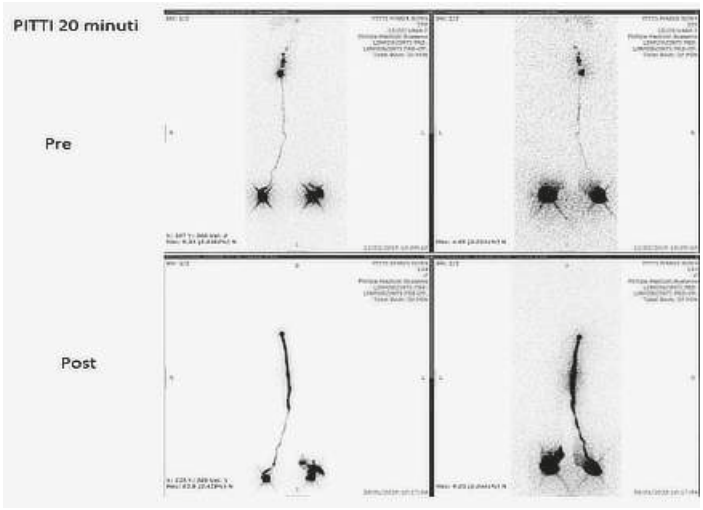
Figure 1: VAS - assessment of the patient's well- being



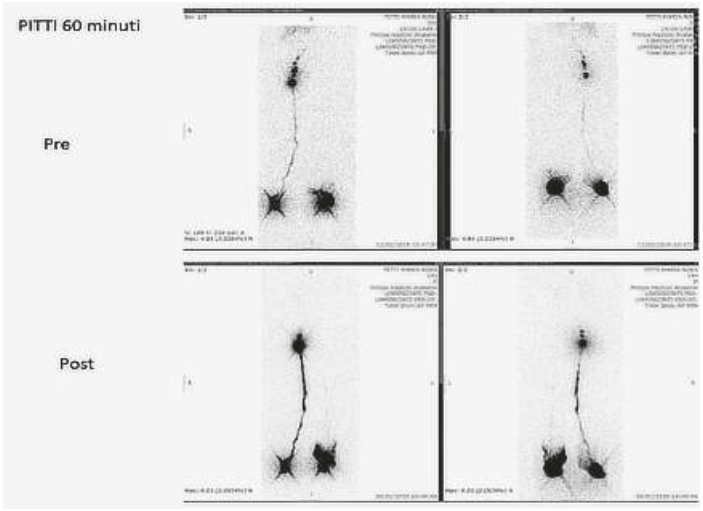
P.M.R. 54 year-old woman, primary lymphedema for 40 years, elephantiasis for lower limbs for 20 years, various pressotherapy and lymph drainage sessions, repeated episodes of lymphangitis.

BEFORE	AFTER
Below Knee: DX 55 cm / SN 65 cm Calf: DX 51 cm / SN 64 cm Ankle: DX 41 cm / SN 48 cm	Below Knee: DX 43 cm / SN 46 cm Calf: DX 41 cm / SN 42 cm Ankle: DX 34 cm / SN 35 cm

Figure 2: Clinical case. Patient PRM



3 A



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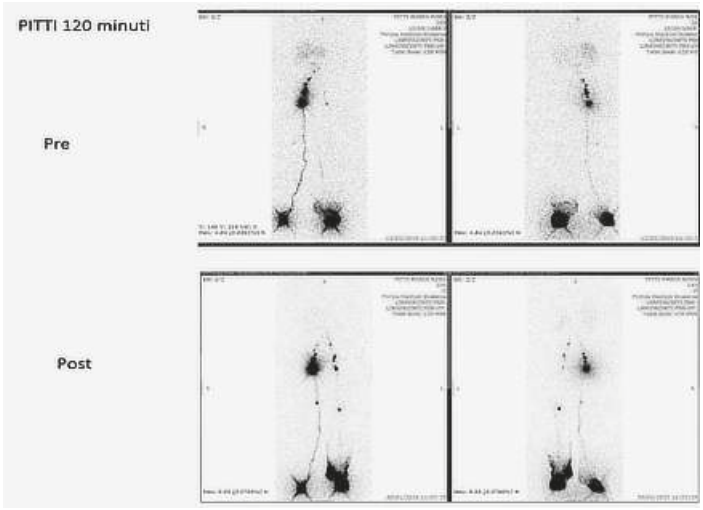
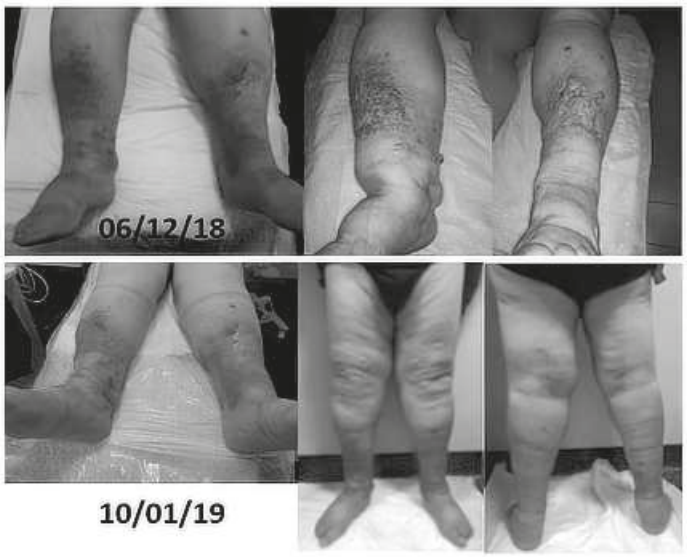


3 C

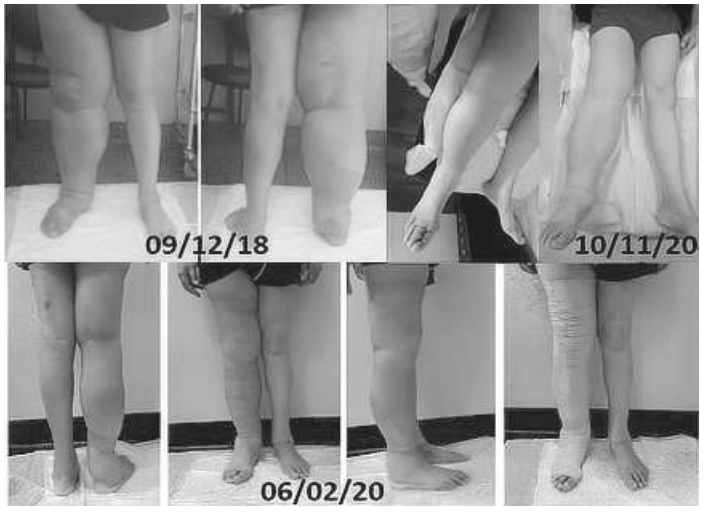
Figure 3: Patient PRM. Lymphoscintigraphy at 20, 60 and 120 minutes (A, B, C), pre and post treatment of PBMNC implantation



N.M. 75 year-old man; Radical prostatectomy; increase in limb volume after about 30 days from surgery; Various pressotherapy and lymph drainage sessions

BEFORE	AFTER
Below Knee: DX 52 cm / SN 54 cm **Calf: DX 53 cm / SN 57 cm** **Ankle: DX 35 cm / SN 33 cm**	**Below Knee: DX 48 cm / SN 51 cm** **Calf: DX 39 cm / SN 44 cm** **Ankle: DX 30 cm / SN 30 cm**

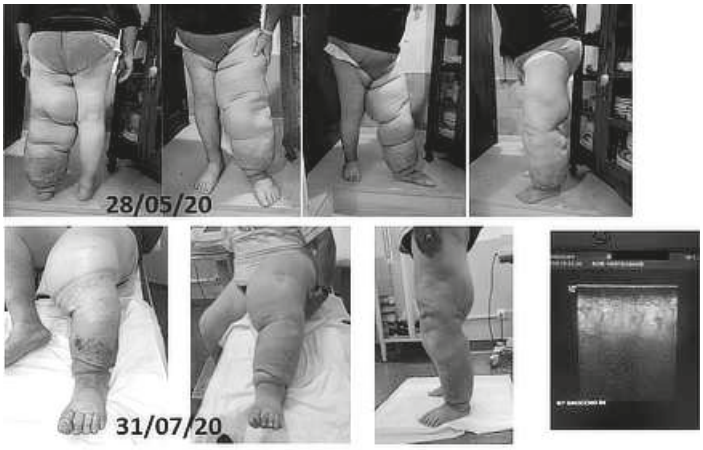
Figure 4: Clinical case. Patient NM



SG: history of primary scrotal lymphedema and repeated episodes of bilateral inguino-crural lymphangio-adenitis; 17 years ago bilateral inguinal lymph node biopsy; about 10 years ago lymphatic-venous anastomosis surgery with persistence and worsening of sinitro lymphedema

BEFORE	AFTER
Thigh: DX 74 / SN / **Calf: DX 57 / SN /** **Ankle: DX 35 / SN /**	**Thigh: DX 55 / SN /** **Calf: DX 40 / SN /** **Ankle: DX 27 / SN /**

Figure 5: Clinical case. Patient SG



S.M. - 74 year - old man
Primary lymphedema for 40 years; Elephantiasis for lower limbs for 30 years; 10 years ago lymphatic surgery; Various pressotherapy and lymph drainage sessions; Repeated episodes of lymphangitis

BEFORE	AFTER
Below Knee: SN 71 **Calf: SN 59** **Ankle: SN 41**	**Below Knee: SN 64,5** **Calf: SN 55,7** **Ankle: SN 38,4**

Figure 6: Clinical case. Patient SB

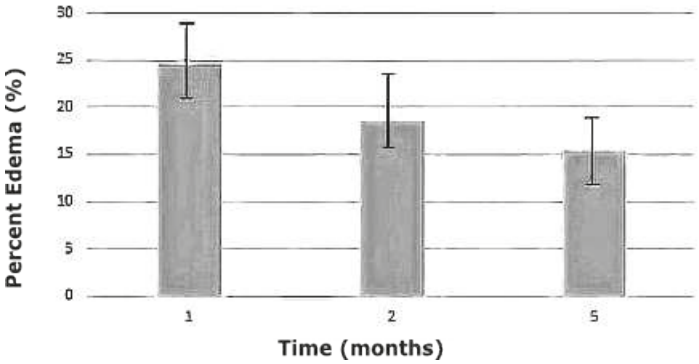


Table 1: Change in percent of edema

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LYMPHATIC-VENOUS MICROSURGICAL BYPASS: HOW, WHEN AND WHY

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Introduction

Among the Lymphatic Surgery techniques, the lymphatic-venous bypass represents one of the most widely used reconstructive microsurgical methods. There are different ways of performing the lymphatic-venous anastomosis but the principle is the same and that is to derive the lymph in the venous stream to by-pass the obstacle to the lymphatic flow. The demolition methods are reserved for the most advanced stages (elephantiasis) and in modern times they can be carried out with minimally invasive procedures. Finally, the LYMPHA technique represents a primary surgical prevention method of lymphedema secondary to lymph node dissection and consists in applying the lymphatic-venous bypass during the same oncological intervention[1-7].

Methods and Techniques

Each of the surgical methods mentioned above has its own indications and contraindications. The lymphatic-venous microsurgical bypass is more indicated in the early stages of lymphatic pathology due to the presence of a minor tissue and lymphatic-lymph node fibrotic component. The lymphatic-venous bypass is based on precise physiological principles starting from the concepts of the mechanobiology of the lymphatic contraction, which are the basis of the peristaltic contractions and releases of the lymphatic collector. Thanks to nerve endings in the lymphatic collector wall, a control mechanism is established for contractions and releases, which occur in a synchronized manner, favoring the active movement of lymph from one lymphatic segment to the next. The other very important aspect for the realization of functioning lymphatic-venous bypass is represented by the pressure inside the venous branch. It is known that venous pressure constantly decreases from the periphery towards the heart. The larger the diameter of the vein in the centripetal direction, the lower the pressure. In the supine position, a peripheral venular pressure of 20 mmHg passes to about 8-12 mmHg in the groin. The pressures obviously increase in standing position.

Comparing the images of a lymphoscintigraphy with Indocyanine Green lymphography, it is possible to understand how a possible lymphatic-venous anastomosis performed distally at the leg or even at the thigh can encounter two types of problems: one is the interruption of the peristaltic wave due to the disruption and distal skeletonization of the collector and the other is the higher peripheral venous pressure, which compromises the lymphatic flow from the lymphatic collector to the venule through the anastomosis itself. Furthermore, a possible occlusion of this peripheral lymphatic-venular anastomosis would lead to the closure of a collector which in any case previously had its own centripetal flow. The conclusion is that it is advisable to perform the lymphatic-venous by-pass as proximally as possible to the site of the obstruction.

As a further confirmation of the latter concept, anatomical findings on the cadaver demonstrate how directly using the collectors that were closed during lymph node dissection, for example in the armpit for a breast tumor or in the groin for another oncological reason, leads to a sort of reopening of the blocked tap with the result of an anastomosis between high lymphatic pressure due to the obstruction and a low pressure vein as a greater caliber and proximal. This procedure leads to a positive lymphatic-venous pressure gradient and in favor of lymphatic drainage. Finally, a possible lymphangitic complication with relative closure and reduced functioning of the anastomosis would not lead to any worsening of the lymphatic drainage of the limb compared to what existed before the surgery, since the collectors used are those that were originally already blocked.

In order to plan the lymphatic-venous microsurgical bypass surgery, an accurate preoperative evaluation is required. The diagnostic tests that are recommended are lymphoscintigraphy, which allows you to fully study the superficial and deep lymphatic circulation of the limb, Indocyanine Green lymphography, which gives information exclusively on the subdermal lymphatic drainage up to about 1 cm below the epidermis, and lymphangio-MRI which is used for chylous dysplasias and for the more advanced stages of lymphedema[8-16].

Results and follow-up

An example of execution of a lymphatic-venous microsurgical by-pass (Fig.1) carried out ... "HOW" ... between brachial lymphatic collectors and a collateral vein of the humeral vein, ... "WHERE" ... proximally, just upstream of the site of the obstacle represented by axillary lymph node dissection, … "WHY"… after a period of at least 6 months of conservative therapy the pathology has not shown a good and stable therapeutic response.

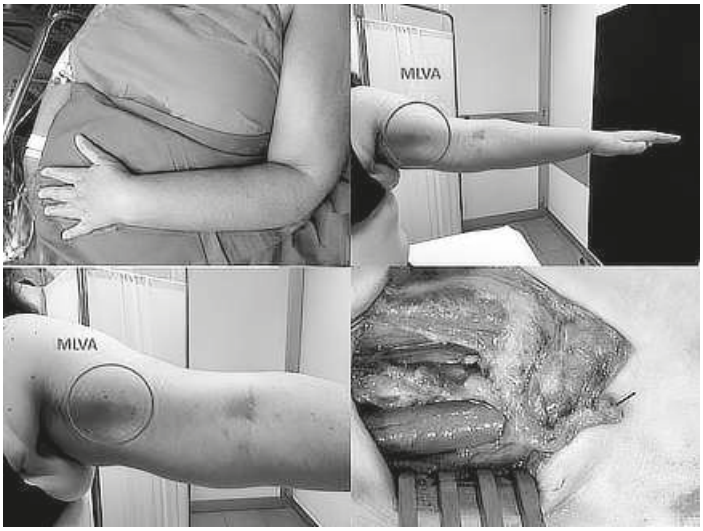

Fig. 1

Figure 2 demonstrates the operative image of the lymphatic-venous microsurgical by-pass at the end and the passage of the ICG demonstrates the patency of the anastomosis itself, through three viewing modes.

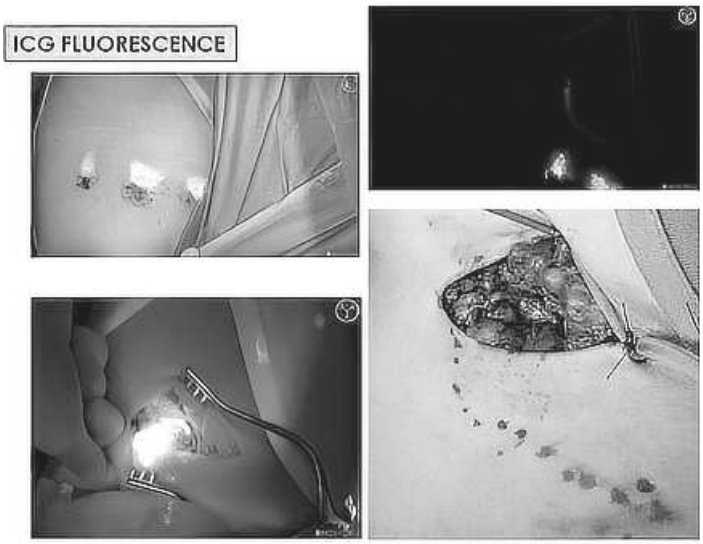

Fig. 2

Figure 3 (A-C) summarizes the concept of the opportunity to perform the lymphatic-venous by-pass as proximally as possible and demonstrates how it is carried out using a vein of congruous caliber and also at a suitable distance from the skin, unlike what is performed peripherally in distal subdermal lymphatic-venular anastomoses, which are immediately below the epidermal layer and which therefore may be affected by compression not only of the surgical scar itself, however small it may be, but also of any other situation that may determine a compressive action on that skin area. Lymphoscintigraphy has proved very useful in evaluating the results at a distance and in this case it highlights a marked reduction in DBF and good patency of the proximal anastomosis.

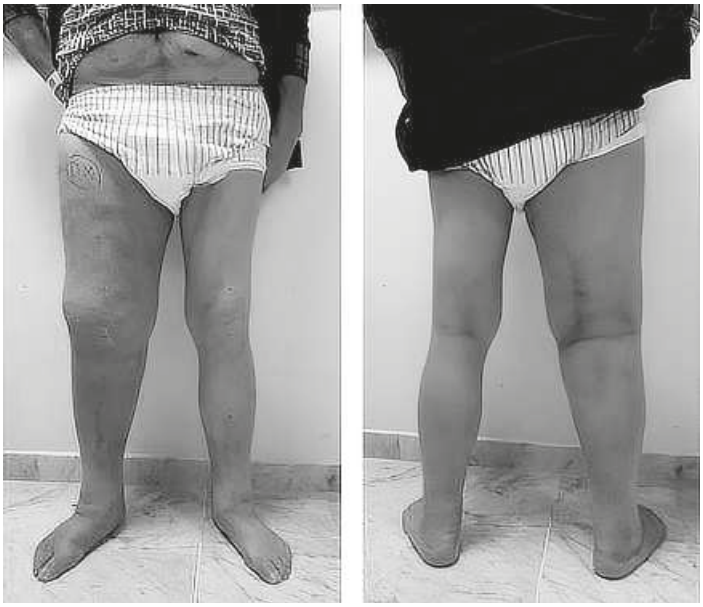

Fig. 3A

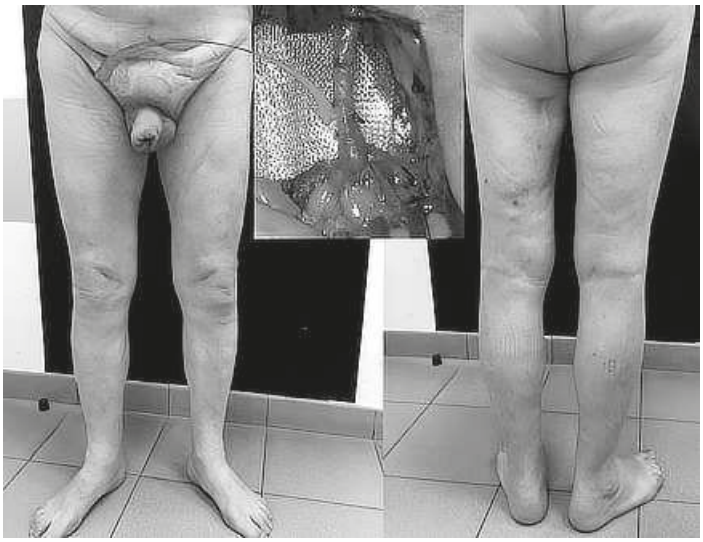

Fig. 3B

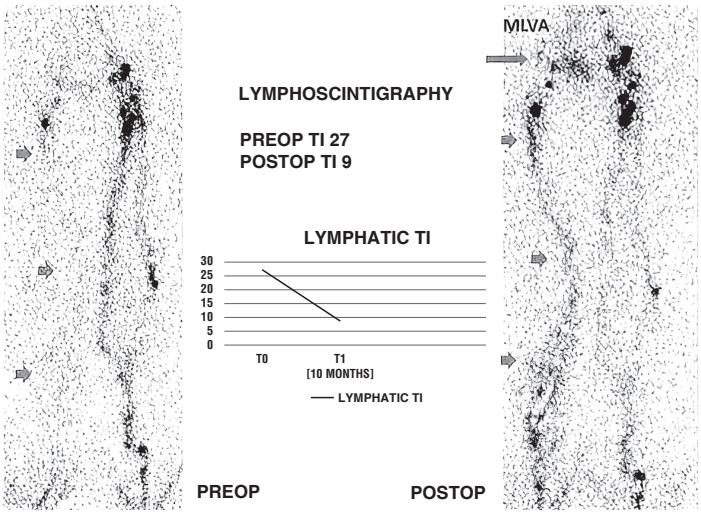

Fig. 3C

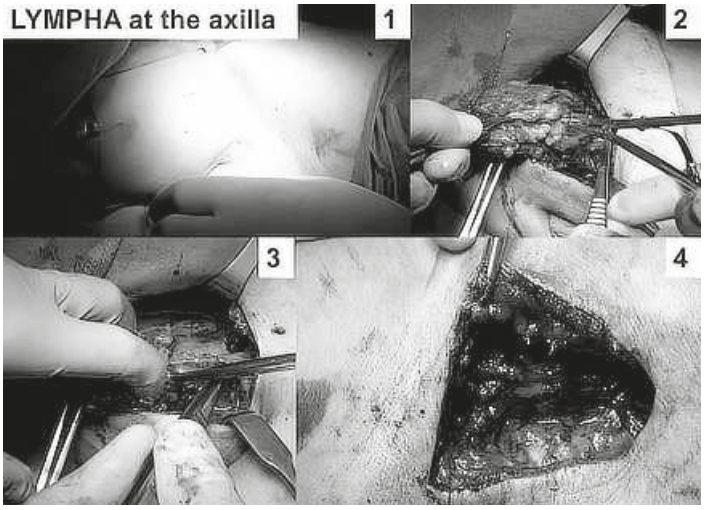

Fig. 4

It is very useful to underline how the advent of indocyanine green functionally effective lymphatic-venous bypasses. The fluorescence used during the surgery not only helps to better track the most functioning lymphatic stalk but also afluorescence has contributed very positively to the creation of llows you to verify the patency of the anastomosis itself. In this type of implementation of the proximal by-pass, Indocyanine green is also injected in depth to visualize even the deepest collectors after having incised the skin and dissected the most superficial layers.

The most important possibility of surgical prevention of secondary lymphedema is represented by the LYMPHA technique, ie the application of the lymphatic-venous anastomosis during oncological surgery. LYMPHA is an acronym which means Lymphatic Microsurgical Preventive Restorative Approach.

Figure 4 summarizes the LYMPHA technique for the prevention of upper limb lymphedema secondary to axillary lymph node dissection in the treatment of breast cancer. Following the numbering, observe the injection of the blue dye and ICG to the arm, the isolation of the lymphatic collectors during lymph node dissection, of a collateral of the axillary vein and the lymphatic-venous bypass at the end of the passage of the dye venous segment. Therefore, instead of closing the lymphatic collectors coming from the upper limb, they anastomose to a vein, to leave the lymphatic tap open and prevent lymphedema of the arm.

The LYMPHA technique is also applicable in axillary dissection for melanoma metastases of the trunk. We also applied the same LYMPHA technique to the lower limb. In case of iliac-obturator and femoral-inguinal lymph node dissection for melanoma metastases of the lumbar region, LYMPHA technique can be associated, ie lymphatic-venous by-pass between lymphatics coming from the lower and collateral limbs of the internal saphenous[17-24].

Conclusions

What then are the conclusive aspects and the main concepts to keep in mind after this presentation on the current role of the lymphatic-venous microsurgical bypass and in particular on how to perform it, when and why. Meanwhile, we can say that the main role of the lymphatic-venous by-pass is that of early treatment and in the prevention of lymphedema, to limit its progression and the appearance of fibrotic tissue and lymphatic-lymph node changes, by means of a multidisciplinary that sees the lymphologist surgeon at the center of a collaboration between general and specialist oncological surgeons.

Fluorescence with ICG has made it possible to significantly improve the short and long term results of lymphatic-venous reconstructive microsurgery and, with particular technical procedures, it also allows to visualize the deeper lymphatic collectors.

The comparison between the volumetric measurements and the pre and postoperative lymphoscintigraphic study made it possible to verify that the distant patency of the proximal lymphatic-venous anastomoses is 97% against 40% of the distal ones and that the best results are obtained by treating lymphedema at the earliest stages.

A very important aspect to keep in mind especially for younger surgeons who are starting this type of surgery is the following: to carry out an effective lymphatic surgery, microsurgical experience alone is

not enough but an adequate lymphological culture is necessary, otherwise the indications and the surgical strategy that are essential for a good final result do not get right.

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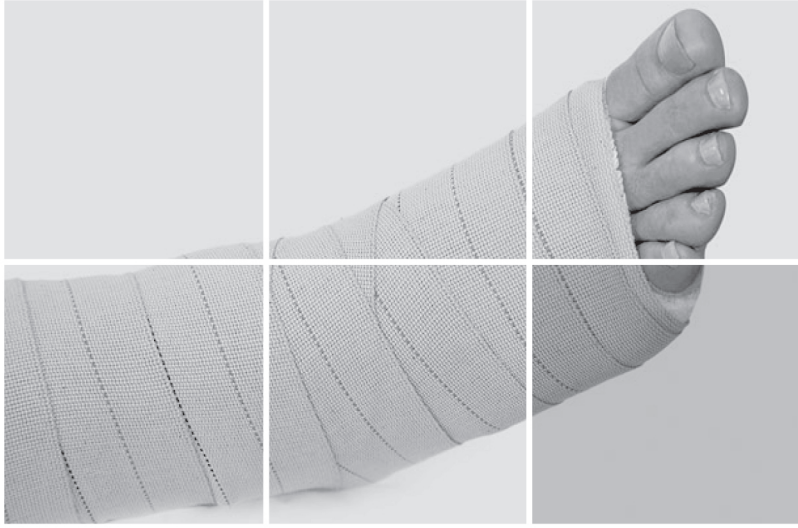

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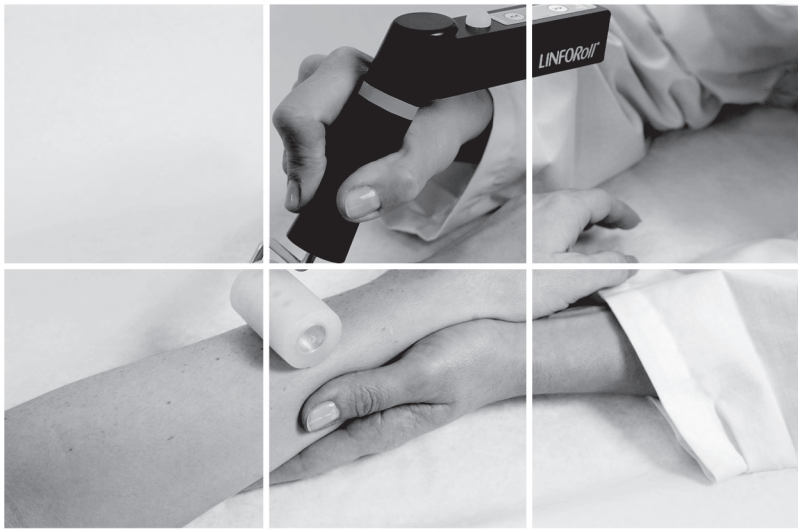

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