THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 21 • No. 61 • 2010
INDEXED IN EXCERPTA MEDICA

SURGERY AND TRANSLATIONAL LYMPHOLOGY: CLINICAL APPLICATIONS IN PREVENTING LYMPHATIC INJURIES

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BACKGROUND

Translation is related to the bi-directional process of applying ideas, insights and discoveries generated through basic science inquiry to the treatment and prevention of human diseases. The challenge consists in integrating molecular insights in Clinical Lymphology, taking to potential advancements from research to clinic and to the community for the benefit of patients. In the same way clinical problems that cannot find proper solutions can be returned to laboratory for further researches.
The problem of prevention of lymphatic injuries in surgery is extremely important if we think about the frequency of both early complications such as lymphorrhea, lymphocele, wound dehiscence and infections and late complications such as lymphangites and lymphedema. Nowadays, it is possible to identify risk patients and prevent these lesions or treat them at an early stage.

Methods. Authors report their experiences in the application of Translational Lymphology in Surgery, pointing out some examples of prevention of lymphatic injuries. After an accurate diagnostic approach prevention is based on different technical procedures. It is very important to follow-up the patient not only clinically but also by lymphoscintigraphy.

Results and Conclusions. It was identified a protocol of prevention of secondary limb lymphedema that included , from the diagnostic point of view, lymphoscintigraphy and, as concerns therapy, it recognized also a role to early microsurgery. It is necessary to accurately follow-up the patient who has undergone an operation at risk for the appearance of lymphatic complications and, even better, to assess clinically and by lymphoscintigraphy the patient before surgical operation.

KEY WORDS: Translational Lymphology, Surgery, Teaching Models, Research, Clinical applications

INTRODUCTION

In the prevention of injuries to the lymphatic system we must consider a primary and a secondary prevention.
Primary prevention includes anamnesis, which aims at pointing out risk factors, such as biological factors (constitution, sex-hormonal status, familiarity, etc.), hygienic, environmental and climatic factors, working activity and the most significant events in remote pathological anamnesis, and protective factors, such as style and habits of life (diet, sanitary measures, sports activity, etc.), social-sanitary culture of Preventive Medicine and epidemiological studies. However, for most of risk and protective factors, mechanism of action are not known yet [1-4].
The rate of occurrence of post-operative complications after inguinal lymphadenectomy reported in Literature is of 6-40% for seromas or lymphocele, 2-4% for haematomas, 17-65% for wound dehiscence, 6-20% for wound infection and 22-80% for lymphoedema [5-7].
The problem of prevention of lymphatic injuries in surgery is extremely important if we think about the frequency of both early complications such as lymphorrhea, lymphocele, wound dehiscence and infections and late complications such as lymphangites and lymphedema. Nowadays, it is possible to identify risk patients and prevent these lesions or treat them at an early stage [8-11].
We tried to perform a protocol of prevention which includes diagnostic investigations such as lymphoscintigraphy and early therapeutic strategies which involve both physical and surgical procedures.

MATERIALS AND METHODS

The main causes of secondary lymphatic injuries are represented by surgical operations, irradiations, infections, infestations (filariasis), traumas, metastatic lymphnodal involvement, etc.
The high risk surgical operations include: radical mastectomy, Wertheim-Meigs operation, oncologic operation in urology, abdominal surgery, lymphadenectomies in “critical sites” (groin and axilla). Other operations are represented by exeresis of lipomas in critical sites, saphenectomy, inguino-crural hernioplasty, etc.
Our experience includes the treatment of a patient with left supraclavicular chylocele due to thoracic trauma, in which we could repair the thoracic duct fistula by microsurgical technique avoiding its surgical closure. In a similar case of thoracic duct fistula, but due to bioptic excision of a supraclavicular lymphnode, we could reconstruct the thoracic duct preventing its ligation. In

another patient with wound infection and lymphangitis at the groin after groin lymphadenectomy due to vulvar carcinoma, we had to excise the infected soft tissues and cover the wide gap with a muscular flap from the rectus abdominis, preserving lymphatics coming from the leg thanks to the blue dye injected at the thigh, to avoid secondary lymphedema of the leg.
Seven patients with pelvic lymphocele were treated by conservative method by putting two drain tubes through the skin guided by ultrasounds and using sclerosing agents and maintaining the tubes in deep aspiration.
Three patints with inguinal lymphocele were treated by removing the lymphocele and performing lymphatic-venous anastomoses to prevent secondary lymphedema of the corresponding limb. In these cases, lymphoscintigraphy is of great help in showing the lymphatic way that fills and maintain the lymphocele. (Figs. 1, 2).
Our widest experience is in the prevention and early treatment of arm lymphedema after breast cancer treatment (189 patients followed-up at over 5 years).
This experience includes the possibility to perform lymphatic-venous anastomoses directly at the same time of axillary lymphadenectomy or very early after breast cancer surgery, following the patient both clinically and by lymphoscintigraphy (Fig. 3).
Another important clinical experience is in the prevention of lymphatic injuries in venous surgery, by using blue dye and preserving lymphatic and lymphnodal structures or performing lymphatic-venous shunts at the same time of the vein surgery, depending on the entity of the lymphatic damage associated with the venous dysfunction.

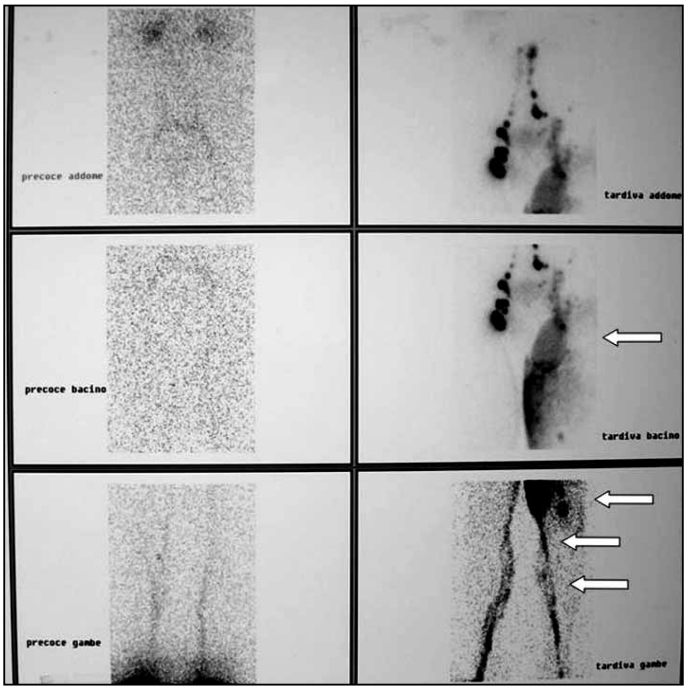

Fig. 1 - Lymphoscintigraphy in a case of lymphocele secondary to groin lymphnodal dissection for a cutaneous melanoma of the right flank. Main lymphatic pathways of the leg fill the lymphocele (arrows).

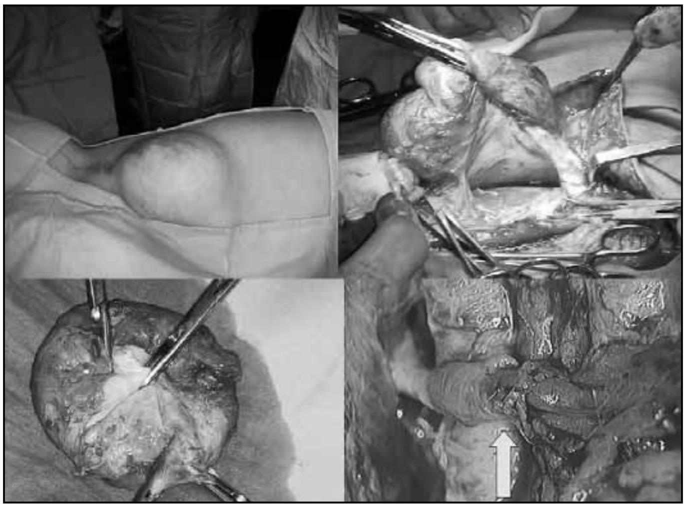

Fig. 2 - The operation of the case reported in figure 1 included the removal of lymphocele and microsurgical lymphatic-venous anastomoses (arrow) between lymphatic of the thigh and a collateral branch of the femoral vein, to prevent the appearance of secondary leg lymphedema.

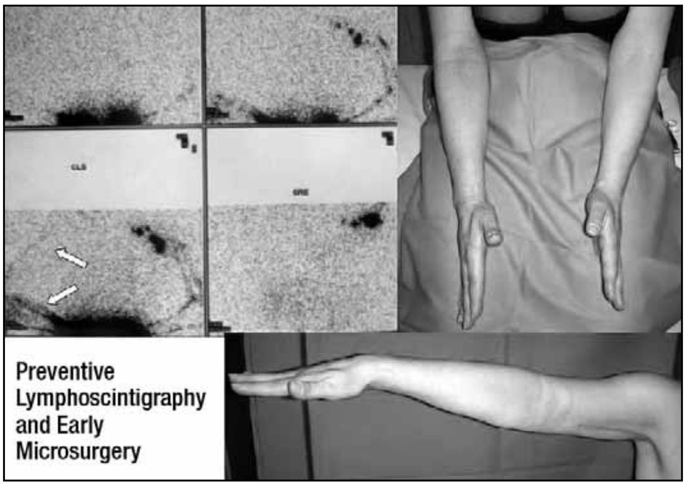

Fig. 3 - Lymphoscintigraphy demonstrated a lymphatic impairment at the right arm in a woman after breast cancer treatment. Lymphedema tended to deteriorate notwithstanding the physical treatment, with also the onset of lymphangitic attacks. Therefore, early lymphatic microsurgery was performed with the complete recovery of the pathology.

In patients with anamnestic potential risk, it is important to educate the patient to examine himself, to visit him periodically and to perform a lymphoscintigraphy. On the other hand, patients without anamnestic risk can be educated to examine themselves and to visit them at distance of time from primary operation. Secondary prevention includes clinical examination and lymphoscintigraphy, which can point out an initial lymphatic impairment before its clinical manifestation (Table 1).

Table 1 - Protocol for the prevention of secondary lymphatic injuries

Primary prevention

Anamnesis
Identification of the risk factors
Identification of the protective factors

Patients without anamnestic risk

Education of the patient to autoexamination
Clinical examination

Patients with potential anamnestic risk

Education of the patient to autoexamination
Clinical examination
Lymphoscintigraphy

Secondary prevention

Clinical examination
Lymphoscintigraphy

Clinical examination *negative*
Lymphoscintigraphy *negative*

Follow up
(6-12 months and once a year for 5 years)

Clinical examination *negative*
Lymphoscintigraphy *positive*

Personal care
Appropriate behavioural changes
Manual lymphdrainage
Follow up

Clinical examination *positive*
Lymphoscintigraphy *positive*

Prevention of lymphangitic complications
Manual and mechanical lymphdrainage, bandages
Elastic compression
Early Microsurgery

RESULTS

Our results were evaluated both clinically and instrumentally (lymphoscintigraphy, ultrasounds, CT, etc.). As concerns the treatment of supraclavicular lymphocele and chylocele, a long term follow-up has shown the complete disappearance and the absence of any relapse of the pathology.
Also ultrasounds and CT scan confirmed the good long term outcome. The lymphatic drainage of patients after groin surgery with lymphatic prevention using the blue dye was investigated by lymphoscintigraphy performed at medium and long term after surgery, and it was demonstrated a good lymphatic sparing and a consequent sufficient lymphatic drainage of the limb.
There was no relapse of pelvic lymphocele even at long term follow-up (over 3-5 years), demonstrating how the conservative treatment of these disorders might be to consider as first choice before a second surgery. The treatment of inguinal lymphocele, removing the lymphocele and performing lymphatic-venous anastomoses to prevent secondary lymphedema was successful. There were no relapse of the lymphocele and there was no appearance of secondary leg lymphedema. Early treatment of arm lymphedema following breast cancer therapy proved to carry out a complete recover of the disease with the stable disappearance of lymphedema and no necessity to wear any compression garment. The same outcome was for the primary surgical prevention of secondary arm lymphedema, performing lymphatic-venous shunts at the same time of axillary dissection.
In venous surgery, results of the use of the blue dye and of lymphatic-venous shunts during surgical operation for varices demonstrated to bring about positive results, in treating patients with both venous and lymphatic disorders or in preventing lymphatic injuries during vein surgery.

DISCUSSION AND CONCLUSIONS

The wide clinical experience above reported in the prevention of lymphatic injuries in general, oncologic, vascular and other specilistic (urologic, gynaecologic) surgery allows us to say that it is nowadays possible to identify patients at low, medium and high risk to develop lymphatic diseases and to prevent them, besides treating lymphatic complications early in order to obtain better results.
The diagnostic investigations that can help us in this sense are represented by ultrasonography, CT scan, MRI, lymphoscintigtaphy and lymphangiography [12-14].
From the operative point of view, it is important to pay attention to lymphatic vessels also during skin incision and to use magnification devices during lymphnodal dissection, using suture material for lymphatic ligations instead of electric coagulation. It is important to use properly the drain tubes, to put them in adequate aspiration associated with proper compression medication on wounds, which might be closed without excessive tension. Proper postural immobilization have to be considered as well [15].
Finally, a proper follow-up of these patients helps in identifying any possible lymphatic complications precociously and thus to treat it very early allowing to overcome the problem completely. This is, therefore, a proposal of prevention and early treatment of secondary lymphatic injuries, which might be applied by all different specialists (general surgeons, urologists, gynaecologists, oncologists, radiotherapists, etc.) during their daily clinical activity to try to get to the aim of preventing the patients, who undergo "risk" operations, from fighting also against complex lymphatic acquired disorders such as lymphorrhea, lymphocele, lymphedema, etc, besides their already more or less serious primary pathological condition.

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VOLUME 21 • No. 61 • 2010
INDEXED IN EXCERPTA MEDICA

LYMPHOSCINTIGRAPHIC QUANTITATIVE PARAMETERS IN PATIENTS WITH LOWER LIMB OEDEMAS: RETROSPECTIVE REVIEW

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ABSTRACT

Aims of the study: To evaluate the sensitivity and the specificity of the lymphoscintihgraphic ‘Kleinhans’ Transport Index (TI) in a general population of patients suffering from lower limb oedema.

Material and Methods: Retrospective study using the medical records and image analysis of 39 patients who have undergone a lymphoscintigraphy for lower limb oedemas (LLE). Patients were divided in 7 groups according to results of the clinical examination, the lymphoscintigraphy and duplex ultrasound investigations. Group A: normal group (n = 23 limbs). Group B: oedema of systemic origin (n = 10 limbs). Group C: Phleboedema (n = 5 limbs). Group D: Latent primary lymph oedema (n = 4 limbs) represented the opposite four limbs in patients with unilateral primary lymph oedema. Group E: primary lymph oedema (n = 12). Group F: secondary lymph oedema (n = 13). Group G: oedema originated from mixed lymphatic-venous insufficiency (n = 8). Kleinhans’ lymphoscintigraphic transport index (TI) were calculated for each group. Sensitivity and specificity of the TI were evaluated with two cut-off values (< 8) en (>15).

Results: In normal group (group A) TI value ranged from 0 to 7 (average 3, standard deviation SD ± 2). In Group B, it ranged from 5 to 19 (average 12, SD ± 7). In group C, it ranged from 3 to 14 (average 7, SD ± 6). In group D, it ranged from 16 to 38 (average 22, SD ± 9). In group E, it ranged from 30 to 45 (average 34, SD ± 8). In group F, it ranged from 25 to 39 (average 32, SD ± 7) and in group G, it ranged from 5 to 22 (average 16, SD ± 11). We have found that TI (with cut-off value <8 needed to define LLE from lymphatic origin or not) can classify correctly the oedematous limbs as lymphatic or not in 67 of 78, i.e. the overall accuracy equal to 85.9%, the sensitivity and specificity were 89.2% (33/37) and 82.9% (34/41) respectively, the positive predictive value and negative predictive value were 82.5% (33/40) and 89.5% (34/38). However, it has a lower specificity 57.7% (15/26) if it applied only to a swollen limb (n = 26), neither with clinical history typical for primary lymph oedema nor a history of previous surgery and/or radiotherapy of the lymphatic system.

Conclusion: The TI appears to have a relatively low specificity in its ability to diagnose patients with lower limb lymph oedema when there is no clinical suspicion of lymphatic origin. Considering that the prevalence of true positive lymph oedematous situation is low, the application of the TI with the proposed threshold value of 7 will lead to one unacceptable high rate of false positive diagnosis of lymph oedematous situations.

KEY WORDS: Lower limb oedema, Lymph oedema, Lymphoscintigraphy, Transport Index.

INTRODUCTION

Lower limb oedema (LLE) is a common manifestation of many disorders and their management requires a correct diagnosis for effective therapy. The differential diagnosis of LLE include lymphatic oedema, oedema due to general systemic disorders (such as heart failure, hypo-proteinemia, liver cirrhosis,…), venous insufficiency and combined veno-lymphatic dysfunction [1]. The lower limb lymph oedema is a syndrome that affect the superficial lymphatic vessels of the lower limb.

Lymphoscintigraphy is the procedure of choice for the diagnosis of lower limb lymph oedema. It is known since the 1950s and has been largely used for the diagnosis of lymph transport disorders of human body [2]. Usually, several morphological and functional criteria are used to describe the status of lymphatic system. Quantitative and semiquantitative methods have also been proposed [3].
Kleinhans *et al.* (1985) were the first authors who used a scoring system to evaluate the lymph transport in patients who underwent lymphatic vessels transplantation. They called it as transport index (TI) [4].
The aim of the present work was to evaluate the sensitivity and the specificity of the Kleinhans' Transport Index (TI) in a general population of patients suffering from lower limb oedema.

ABSTRACT

Lymphoscintigraphic procedure

A Three-phases Lymphoscintigraphy is performed. The first phase (Rest phase) begins after subcutaneous injection of 99mTc nanocolloid 0.2 ml (size 40-80 nm) equivalent to ~55 (range 50-60) MBq in the first interdigital web space of each lower limb. Using a gamma camera with low energy high-resolution (LEHR) collimator (matrix of 128x128), imaging of the sites of injection (acquisition times: 5x60 sec) were first obtained, then followed by dynamic imaging at the level of the lower legs for 15 minutes, of the knees for 15 minutes and finally of the inguinal areas for 15 minutes. Immediately thereafter, Phase 2 (Stress Phase) starts during which the feet of the patient are connected to a foot dynamo for passive movements for 15 minutes and with gamma camera acquisition at the level of the inguinal areas (acquisition time 15x60 second). The third phase (Late phase) is obtained after 1 hour walking. Static imaging are taken (2 minutes long) at the level of the lower legs, knees, thighs and inguinal areas.

Classical analysis of the lymphoscintigraphic data

We have chosen the criteria described by Nawaz *et al.* (1985, 1990) and revised by Weissleder *et al.* (1988) (5,6,7) to discriminate between normal or abnormal lymphoscintigraphies in order to find our population groups. These criteria include:

- Early proximal migration of tracer along draining lymphatics (appears as single or double medial bands);
- Visualization of pelvic lymph nodes (para-iliacal, para-aortic and lever on delayed image (i.e.120 minutes) bilaterally, symmetrical and equal in number and size;
- Stasis or subcutaneous pooling of tracer or dermal backflow;
- Absence of medial bands along the lower leg and thigh with lymph oedema;
- Non-visualization of inguino-pelvic LN on delayed images;
- Visualization of deep lymphatic channels on medial side of the leg;
- Visualization of collaterals;
- No movement of the tracer from the injection site.

The combination of the functional and morphological data lead to a classification of the lymphoscinbtigrams as normal, as diagnostic for lymphatic oedema and as diagnostic for combined veno-lymphatic ("mixed") oedema. Table 1 summarize these criteria.

The kleinhans Transport Index

Kleinhans *et al.* described in 1985 a scoring system to evaluate the lymph transport kinetics calling it the transport index (TI). This score represent a summation of a numerical grade to every parameter (T, K, D, N and V) where:

T = 0.04 n (n: Time in minutes to the first appearance of regional lymph nodes).
K = Lymphatic transport kinetics
D = Distribution Pattern
N = Assessment of Lymph nodes
V = Assessment of lymphatic vessels

The calculated formula proposed by Kleinhans *et al.*:

$$TI = T+K+D+N+V$$

Those grades are linked to the parameter and shown in table 2. The resulting transport index (TI) ranged from 0 (completely normal) to 45 (completely pathological). According to the results of Kleinhans *et al.* this score is considered as abnormal if it >7 [4].

Table 1 - Summary of diagnostic conclusion according to functional and morphological patterns of lymphoscintigraphic images.

Diagnostic conclusion	Functional pattern	Morphological pattern
Normal	Normal pattern	Normal transport of lymph and appearance of inguinal lymph nodes
Lymphatic oedema	Obstructed pattern	Absent or very little lymph transport, no appearance of inguinal lymph nodes, dermal backflow
Combined Veno-lymphatic eodema	Mixed pattern	Dilated lymph vessels, increased visualisation of inguinal lymph nodes, rapid transport into systemic circulation

Table 2 - Grade (0, 3, 5, 9) linked to a parameter (K, D, N and V). See text.

Grade	0	3	5	9
Parameter				
K	No delay	Low grade delay	Extreme delay	Missing transport
D	Normal distribution	Partial distribution	Diffuse distribution	Transport stop
N	Clearly demonstrated	Faint visualization	Hardly recognizable	No visualization
V	Clearly demonstrated	Faint visualization	Hardly recognizable	No visualization

To use this table, you have to add every grade of a parameter to each other. For example, the time of appearance of the first regional lymph node after injection = 0.04 x 45 = 1.8, the grade of the parameter missing transport (K) = 9, the grade of diffuse distribution (D) = 5, the grade of hardly recognizable lymph node (N) = 5 and the grade of no visualization of lymphatic vessels (V) = 9. Thus the resulting TI ~ 30.

RESULTS

1) TI analysis according to the different groups

From 01/01/2003 until 01/01/2005, 39 patients (35 females and 4 males) were referred because they were complaining of swelling of one or both lower limbs (51 clinically oedematous limbs standing from 4 to 24 months). Lymphoscintigraphic investigations were retrospectively reviewed and analyzed taking into account to clinical data, duplex ultrasound examinations of the lower limbs together with the results of our lymphoscintigraphic procedure. Oedematous limbs were classified in 7 groups according to these data (see table 3).

A) Normal Group

This group comprise 23 lower limbs, without oedema, with normal clinical examination, normal morphological and functional lymphoscintigraphic results and normal duplex ultrasound examination. The TI ranged from 0 to 7 with average of 5,5 (SD: 1,5).

B) Group of systemic oedema

This group comprises 5 patients with clinical bilateral lower limb oedema with both normal duplex ultrasound and lymphoscintigraphic morphological and functional assessment. The TI range from 5 to 19 with average of 12 (SD±7). Two limbs had a TI value higher than 15.
One patient had TI below 7 for both limbs. Thus TI was abnormal in 4/5 patients (8/10 oedematous limbs).

C) Group of pure venous insufficiency

Eight lower limbs with abnormal clinical examination had obvious venous insufficiency on duplex ultrasound examination of the lower limb with normal lymphoscintigraphic (morphologic and

Table 3 - The range of TI in relation to the groups of oedematous limbs.

Group	Clinical examination	Total No. of limbs	No. of limbs with normal TI*	No. of limbs with abnormal TI*	Transport Index (TI) Range (average ± SD)
Normal limbs	Normal	23	23	0	0.2-7 (5,5±1,5)
Systemic oedema	LLE	10	8	2	5-19 (12±7)
Pure venous insufficiency	LLE	8	3	5	3-14 (7±6)
Latent primary LE	Normal	4	0	4	16-38 (22±9)
Primary LLE	LLE	12	0	12	30-45 (34±8)
Secondary LLE	LLE	13	0	13	25-39 (32±7)
Combined V-L LLE	LLE	8	4	4	5-22 (16±11)

(*) TI is considered abnormal if higher than 7.

functional) assessment. The calculation of the TI showed a relative higher reading: the range is 3-14 with average of 7 and SD ± 6.
Three of the 8 Lower limbs have TI below 7. One patient with bilateral lower limb oedema where TI value 3 and 5. Another patient have unilateral lower limb oedema where the calculated TI is 5 and the contralateral lower limb is normal. (i.e. the TI is abnormal in 5/8 oedematous limbs).

D) Group of latent lymph oedema

Four patients with primary lymph oedema affecting one lower limb had no clinically obvious oedema at the level of the contralateral limb but the lymphoscintigraphy showed impeded lymph transport at rest which is normalized at stress. The TI ranges from 16 to 38 (average is 22 SD± 9).

E) Group of clinical (overt) primary lymph oedema

This group comprises of 8 young women with mean age: 25± 4 years. Clinically swollen limbs were unilateral in 4 patients and bilateral in 4 patients (there were 1 patient with Klippel-Trenanuny syndrome and bilateral lymph oedema). The lymphoscintigraphy showed obstructed functional pattern. Morphologically the oedematous limbs (12 lower limbs) showed absence of lymph transport during all phases of the lymphoscintigraphy. The TI ranged from 30 to 45 (average calculated TI is 34 and SD is ± 8).

F) Group of clinical secondary lymph oedema

This group comprises 11 patients (10 females and 1 male: average age 53 ± 4) in 13 lower limbs. The oedema was unilateral in 9 patients and bilateral in 2 patients (with bilateral inguinal lymph evidement for pelvic cancer: vulva carcinoma and penile carcinoma). They were regarded as secondary because of a clinical history of involvement of lower limb lymphatic system in a surgery (most common : radical operations for genital cancer, or due to trauma of the limb Lower limb). The lymphoscintigraphy of the affected limb showed obstructed functional pattern and morphologically there were prominent dermal backflow, lymph transport stop at site of injury or operation.
The TI ranged from 25 to 39 (average 32 and SD is ± 7).

G) Group of combined veno-lymphatic oedema

Four patients had bilateral lower limb oedema (8 lower limbs) with abnormal duplex ultrasound and clinically apparently venous oedema with abnormal lymphoscintigraphy. The TI ranged from 5 to 22 (average 14 and SD is ± 11). Four limbs had a TI value higher than 15.

2) Analysis of the TI sensitivity and specificity

A shown in Table 4, the TI correctly classified the oedematous limbs as lymphatic or not in 67 of 78, i.e. the overall accuracy is equal to 85.9%. The sensitivity and specificity of the TI in our population are 89.2% (33/37) and 82.9% (34/41) respectively. Positive predictive value (PPV) and negative predictive value (NPP) were 82.5% (33/40) and 89.5% (34/38) respectively.

Table 4 - TI results (cut-off = 7) versus the lymphatic or not lymphatic origin of oedematous limbs in the whole series.

	Lymphatic Oedema	No Lymphatic Oedema
TI>7	33	7
TI<8	4	34

However, if TI is applied to patients with swollen limbs (n = 26), either without clinical history typical for primary lymphatic origin, or without previous surgery and/or radiotherapy of the lymphatic system, the specificity of the TI drops to 57.7% (15/26) (see table 5).

Table 5 - TI results (cut-off = 7) versus the lymphatic or not lymphatic origin of oedematous limbs in patients without clinical history either of secondary lymphatic oedema, or of primary lymphatic oedema. To increase the specificity of the TI, we proposed a threshold value equal to 15 and the specificity will be 21/26 or 80.5%.

	Lymphatic Oedema	No Lymphatic Oedema
TI>7	0	11
TI<8	0	15

If we increase the TI threshold up to 15 , the overall accuracy of the test would be increased up to in 68 of 78 i.e.87.1% discriminating the oedematous limbs as lymphatic or not in. However, the sensitivity value will also increased up to 100% (35/35) but the specificity value will be decreased 75% (33/44) (see table 6).

Table 6 - TI results (cut-off =15) versus the lymphatic or not lymphatic origin of oedematous limbs in the whole series. This would emphasize that any increase in our cut-off value of TI would result in increased sensitivity on expense of specificity: i.e loss of differentiating power of the test between the types of lymph oedema.

	Lymphatic Oedema	No Lymphatic Oedema
TI>15	35	9
TI<16	2	33

DISCUSSION

Lower limb oedemas represent a relatively frequent clinical problem. The differential diagnosis of LLE include oedema due to general systemic disorders (such as heart failure, hypoproteinemia, liver cirrhosis,…), venous insufficiency, lymphatic dysfunction (either secondary, or primary) and combined veno-lymphatic dysfunction. In most of the cases, the clinical history and the clinical examination of the patient will lead to the correct diagnosis. On the other hand, the venous problems will be easily diagnosed using duplex ultrasound. Most of the lower limb oedemas of lymphatic origin can also be diagnosed based on their clinical history (previous surgery for secondary lymph oedemas or typical appearance in young adults for primary lymph oedemas). Nevertheless, many patients come with "heavy limbs", limited oedemas also sometimes transient, with normal and/or abnormal venous investigation and then the diagnosis of lymph oedema, of the lymphatic insufficiency becomes difficult .
Lymphoscintigraphy is the procedure of choice to confirm this diagnosis [8,9]. The contribution of sole morphological imaging has been proven to be insufficient to establish the diagnosis of lymph oedema [10]. Morphological abnormalities of the limb lymphatic system (dermal backflow, collateralization through superficial subcutaneous lymphatic, lymphatic blockades, absence of lymph node visualization, demonstration of deep lymphatic system) have been reported in up to 70% of the cases [9,10]. Thus the sensitivity of lymphoscintigraphic investigation protocol is only optimized when all data, quantitative as well as morphological and functional are taken into account [10]. In our series, lymphoscintigraphy excluded lymphatic insufficiency as a cause of leg swelling in more than half of the examined lower limb (groups A, B and C).
In patients with lower limb oedema attributed to an underlying systemic diseases (heart failure, hypoalbuminemia… etc), the lymphoscintigraphy is normal morphologically and functionally although sometimes haemodynamically aberrant. Balance between the hydrostatic and oncotic pressures which control the flow of interstitial fluid from cellular and extracellular compartments to the vascular compartments include the lymphatic drainage. In group C the duplex ultrasound examination showed abnormal venous component of the lower limb which is concomitant with the clinical examination. We observed that pure venous insufficiency show a normal morphologic and functional lymphoscintigraphic pattern. In case of latent lymph oedema (group D), the clinically non oedematous limbs from the patients with primary lower limb lymph oedema showed great variation in their morphological and functional lymphoscintigraphic assessment. At the level of these limbs, lymphoscintigraphy showed nearly an obstructed pattern. This proves that the non-overt clinical oedema in patients with primary oedema has abnormal lymphatic structures and it might end in a clinically obvious lymphatic oedema sooner or later. This can be associated in general management and prompt treatment of the patient. In patients with clinical diagnosis of primary or secondary lymph oedema, lymphoscintigraphic investigations were always confirmatory [9,10]. In cases of combined veno-lymphatic dysfunction, the most affected limb showed an enhanced pattern with morphological appearance of dilated lymph vessels, increased visualisation of the inguinal lymph nodes and rapid transportation into systemic circulation (liver).

Kleinhans *et al.* proposed in 1985 a numerical scoring system reflecting morphological and functional results of the lymphoscintigraphy. They called it transport index (TI) [4]. Several authors reported sensitivity and specificity values for this index to be higher than 90% [4,9,13,14]. When applied to our population, the TI correctly classified the oedematous limbs as lymphatic or not in 67 of 78, ea one overall accuracy equal to 85.9%. The sensitivity and specificity of the TI in our population are 89.2% (33/37) and 82.9% (34/41) respectively. PPV and NPV were 82.5% (33/40) and 89.5% (34/38) respectively. However, if TI is applied to patients and/or limb oedemas (n = 26), neither with clinical history typical for one primary lymphatic origin, nor with previous surgery and/or radiotherapy of the lymphatic system, the specificity of the TI drops to 57.7% (15/26). The false positive rate is thus 42.3%.
In fact, the low specificity that we found in these last patients – although based on small series – might raise the question of the use of the TI in a general population with "heavy limbs", "limited and transient lower limb oedemas". To increase the specificity of the TI, we proposed a threshold value equal to 15 and the specificity will 21/26 or 80.5%.

As an explanation of these results, the lymphoscintigraphy procedure is highly methodology dependent, it differs from hospital to another by use of different type of tracer, site of injection and route of administration [17-23]. Also the TI is greatly dependent on lymph kinetics, type of injection (subcutaneous or intracutaneous) and on site of injection [4,14]. Here we have found that the type of tracer may also play a major role in variability of this index even in normal cases. In their initial paper, Kleinhans et al used stannous sulfur colloids labelled with 99m Tc (110 MBq) with particle size from 20nm up to 60nm. They injected 0.5 ml subcutaneously in the web space between 1st and 2nd digit. Mobilization of the extremities was done by ergometric stress (20W for 3 min) [4]. Although using the same index, Cambria *et al.* used 99mTc labelled antimony trisulfide colloid injected subcutaneously into the second interdigital space. The feet were connected to a foot dynamo for exercise [9]. Interpretation of the lymphoscintigrams represents also the critical point of the procedure [10]. Indeed, the sole objective parameter in the Kleinhans' formula is represented by the time in minutes to the first appearance in regional/inguinal lymph nodes. All other parameters, K, D, N and V, may be subject to the subjectivity of the "readers". For example, we do not find a difference between (K: lymphatic transport kinetics) and (N: assessment of lymph nodes).
In our procedure, we used 99mTc-labeled nanosized human serum albumine (HAS) colloid particle with size (40-80nm) [15]. From clinical considerations, many oedemas only appear when limbs are in resting conditions (so called dependent oedema) and may disappear with exercise, implicating a degree of lymphatic valvular, or vascular insufficiency [8,10,15]. Pathophysiologically, the disappearance of the tracer injected in resting conditions during the first phase of the examination is mainly determined by a loco regional status of the initial and primary collectors (the lymphatic vessels) and by differences in hydrostatic and oncotic pressures between the interstitial tissue and the lymphatic vessels [16]. During the second phase of the examination (the stress phase), the

exercise (tip-toeing) involve additional mechanisms: the active opening of the initial lymphatics, the local increase of arterio-venous flow and the activation of contraction of the smooth muscles covering the lymphatic endothelium. On the another hand, oedema may only appear when the patient is standing up or seating and after a variable period of normal activity, what is the justification of our delayed imaging taken one hours after injection.

CONCLUSION

Initially designed as a numerical value for the follow up patients with lymphatic transplantation, the TI proposed by Kleinhans *et al.* appears to have a relatively low specificity in its ability to diagnose patients with lower limb lymph oedema when there is no clinical suspicion of lymphatic origin. Considering that the prevalence of true positive lymph oedematous situation is low, the application of the TI with the proposed threshold value of 7 will lead to one unacceptable high rate of false positive diagnosis of lymph oedematous situations.

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THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 21 • No. 61 • 2010
INDEXED IN EXCERPTA MEDICA

THE ROLE OF DIAMAGNETIC PUMP (CTU mega 18) IN THE PHYSICAL TREATMENT OF LIMBS LYMPHOEDEMA. A CLINICAL STUDY

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INTRODUCTION

Lymphoedema represents a chronic pathology, that renders patients physically and psychologically disabled, it is not easy to control, and shows a marked tendency to spontaneously set in complications. For such reasons lymphoedema demands for a novel early, targeted and lasting diagnostic and therapeutic approach [(1)].

So far it is frequently claimed, in a completely misleading manner, that nor the lymphoedema physiopathology is clear or the corresponding treatment is satisfactory. Nevertheless, though the pathogenetic details are still an open question, the general principles of the disease’s physiopathology are well known.

On one side, the main disorder may be characterized by a **“low output failure”** of the lymphatic system, that is, a general decreasing of the lymphatic flow. Such a disorder can be due to a congenital lymphatic dysplasia (primary lymphoedema) or to an anatomic obliteration, for example caused by a radical surgical resection or by radiotherapy, or again as a consequence of repeated lymphangitis with lymphangiosclerosis or, finally, produced by a functional insufficiency such that due to lymphangiospasm, paralysis and valvular insufficiency (secondary lymphoedema). In any case, the common feature is a disorder in the lymphatic transport mechanism, that decrease below the minimum capability required by the microvascular filtrate, that includes plasmatic proteins and cells that normally come out from the haematic network entering the interstice.

On the other side, the **“high output failure”** of lymphatic circulation occurs when an excess of capillary haematic filtrate overcomes the normal transport capability of the lymphatic system as for example happens in the liver cirrhosis (ascites), in the nephrosic syndrome (anasarca) and in the inferior limbs deep venous insufficiency (post-thrombophlebitic syndrome) and the severe phlebostasis [(2)]. The lymphatic injury, both primitive and secondary, worsen in time due to the creation of a vicious circle: LYMPHATIC DISORDER → INCREASE OF PROTEIN RICH INTERSTITIAL LIQUID → DECREASE OF PROTEOLYTIC CAPABILITY → INCREASE OF INTERSTITIAL CONNECTIVE → FIBROSIS [(3)]. In the subcutaneous tissue of patients affected by lymphoedema an increase of the amount of interstitial liquid, rich in proteins,is observed. To the increase a chronic phlogosis is associated (the monocyte-macrophage system and the fibroblast are activated), with a growth of the interstitial matrix. The lymph accumulates in the fascia, forming “holes” or “lymphatic lakes” and the three-dimensional retinaculum structure addresses molecules and lymph toward the cutis surface. The hydrophobic adipose lobules keep the water component off, so that it accumulates along the retinaculum. Finally, we observe an upsetting of the subcutaneous tissue, with the appearance of “comb” picture [(4)].

PULSED LOW-FREQUENCY ELECTROMAGNETIC FIELDS: The pulsed low-frequency (< 50 Hz) electromagnetic fields [(5)] belong to he class of non ionizing radiations, that is, they are characterized by an associated energy below 12 eV (electron-Volt). Such an energy is insufficient both to turn on ionization phenomena in molecules and to break even very weak chemical bonds. For this reason in the last decades these radiations have not been considered able to interact with biological systems and, as a consequence, the studies on this subject were scarce and information poor, especially when compared with the great amount of knowledge concerning the interactions among ionizing radiations and biological systems [(6)]. Only recently, due to the more and more common use of electromagnetic fields of different intensity and frequencies [(7)], a vast research activity [(8-9-10-11-12-13-14-15)] has started, addresses to the definition of their main biological and therapeutic effects, on which are based the exposition thresholds currently recommended (Tab. 1):

Table 1.

	Types of Radiation	Frequency	Wave Length	
Non-ionizing Radiation	Lowest Fields fz	0 Hz - 50 Hz	Over 6000 Km	–
	DIA System Low Frequency Electromagnetic Fields	50 Hz - 100 Hz	Over 3 Km	Dangerousness of Radiation
	Magnetic Therapy High Frequency Radiations	100 KHz - 300 GHz	1 mm - 3 Km	
	Radio Transmission Marconi Therapy / Radar Therapy Infrared	> 300 GHz	780 nm - 1 mm	
	Fisiotherapic treatment Visible light		380 nm - 780 nm	
	Phototherapy treatment Ultraviolet rays		10 nm - 380 nm	
	Tanning treatment		Under 10 nm	
Ionizing Radiation	X rays and Gamma radiation **Clinical diagnosis / Biological sterilization**			+

DIAMAGNETISM: The diamagnetism works on hydrogen atoms. Indeed, when a hydrogen atom is covalently bound to a strongly electronegative atom, as for example the oxygen, the bond electrons tend to move toward the latter. As a consequence, the H atom assumes a partial but consistent positive charge. This charge, distributed in a small volume, lead to a high electric charge density. At this point, the hydrogen atom tends to bind with a partially negatively charged atom (the oxygen atom of a different water molecule) in this way acquiring a greater stability neutralizing its electric charge (Fig. 1)

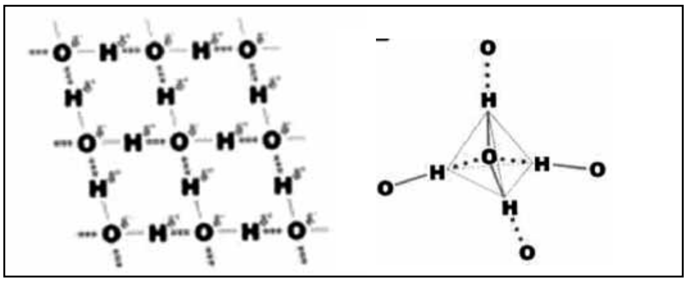

Fig. 1

A single water molecule does not feel any net force, since it is subject to the action of the surrounding molecules that are uniformly distributed in any direction of the three-dimensional space. The liquid water consists in a disordered network of molecules, bound together by relatively weak chemical bonds. Such a network is continuously subject to fluctuations that randomly break and create new bonds among the molecules. Due to these characteristics the water does not have a proper dipole magnetic moment and it is repelled by an external magnetic field (**diamagnetism**). The CTU Mega 18 is a device of molecular diamagnetic acceleration. It uses an energy of up to 200 Joule, generating high power (2 Tesla) pulsating fields and developing a water-repulsive force with the following main therapeutic aims:

– liquids transport;
– implant of pharmacologically active hydrosoluble molecules, bound to the water (salvation water);
– tissue biostimulation.

Liquids transport: As a result of diamagnetic repulsion, the free water in the extracellular compartments is fiercely pushed away from the field application site. The transport of extracellular liquids helps the oedema and post-traumatic effusions reabsorption and the scoriae removal, and stimulate the lymphatic circulation and related phenomena also thanks to the vasodilatation draining action produced by the diathermia coupled with CTU Mega 18. In addition, the magnetic field works on the intracellular liquids, increasing their mobility. The increase of the thermal molecular excitation supports the cells biochemical activity as well as the mitochondrial and phagic-lysosomal metabolic mechanisms. The result is a beneficial acceleration of all energetic, metabolic and cellular activities like ionic transport, scoriae removal and cellular breathing.

Implant of pharmacologically active molecules: The process works according to the principle of diamagnetic force that exerts a repelling force on the water. The handling can be obtained through a dilution in water (salvation water). Indeed, the magnetic fields do not produce effects of cutaneous polarization working as diffusion walls that otherwise appear with the use of devices operating with electric currents (ionophoresys, electroporation, etc.). This permits a deeper action and a better uniformity in the drug distribution with respect to traditional systems.

Tissue biostimulation: A variable magnetic field crossing a conductor induces an electric current. The human body is a conductor, that when it is crossed by a magnetic field the phenomenon of biostimulation occurs. The action of magnetic fields is well described in terms of bioelectric parallelisms existing among cells [17], since it acts on the difference of electric potential on the membrane sides as well as on the orientation af the circulating atoms that behave as elementary magnetic dipoles [18-19].

* * *

The CTU-Mega 18 posses energetic transfer supplier for capacitive and resistive diathermia, that permits to produce a thermal effect having the following main characteristics:

– action on the microcirculation;
– action on the adipocytes;
– action on the mucopolysaccharidic gel;
– action on the interstitial weft.

Action on the microcirculation: The diathermia stimulates a microhyperaemia that permits to overcome the artery-arteriolar deficit and to increase the flow speed in capillaries. In this way the microcirculatory stasis is fixed and the interstice oedematous flooding [20].

Action on the adipocytes: The recovery of the microcirculation raises the thermal and enzymatic gradients, in this way reactivating the lipolysis. In addition, the friction caused by the shift currents of ionic charges moving in the tissue produces a localized and homogeneous increase in temperature, that restores the normal turn-over needed to maintain an active adipocytes metabolism.

Action on the mucopolysaccharidic gel: The diathermia returns the right fluidity to the gel both operating on its components and restoring the membrane selectivity that tunes the osmosis among the endoluminal, vasal and interstitial compartments. Such activity is further on magnified by the effects of the magnetic fields, thus justifying the improvement of the matrix “gel-sol” transition.

Action on the interstitial weft: The diathermia, while increasing the temperature, induces an increase in the macrophagic proteolytic activity, in this way reducing the compactness of the connective fibres with a consequent decrease of echogenicity [21].

MATERIALS AND METHODS

In the framework of the Vascular Surgery Operative Unit – University of Ferrara and of the “Oedema Centre” in Nola (NA) we evaluated 42 limbs affected by lymphoedema in 38 patients aged from 21 to 67 (average 47 ys). 34 patients were affected by monolateral lymphoedema (30 in the inferior, 4 in the superior limb) and 4 by bilateral lymphoedema, with oedema localized at inferior limbs. We built up two randomized groups. Patients in group 1 were treated with CTU Mega 18 together with 2nd class compression stockings; patients in group 2 were treated only with 2nd class compression stockings.
All patients were clinically evaluated using a standard procedure we proposed, see Table 2 [23], before recruiting, by an accurate clinical examination and instrumental exams (lymphoscintigraphy, soft tissues echography, echodoppler). In addition, the limb’s circumference was measured in specific positions before and after the treatment (60 days).
Every patients were asked to fill an Inform and Consent form and submitted to an accurate case history interview using a clinical file devoted to CEAP-L Classification [23] that permitted us, at the end of the study, to get objective clinical conclusions. Moreover, specific exclusion causes to the diamagnetic pump treatment (epilepsy, peace makers, MR incompatible metallic component).

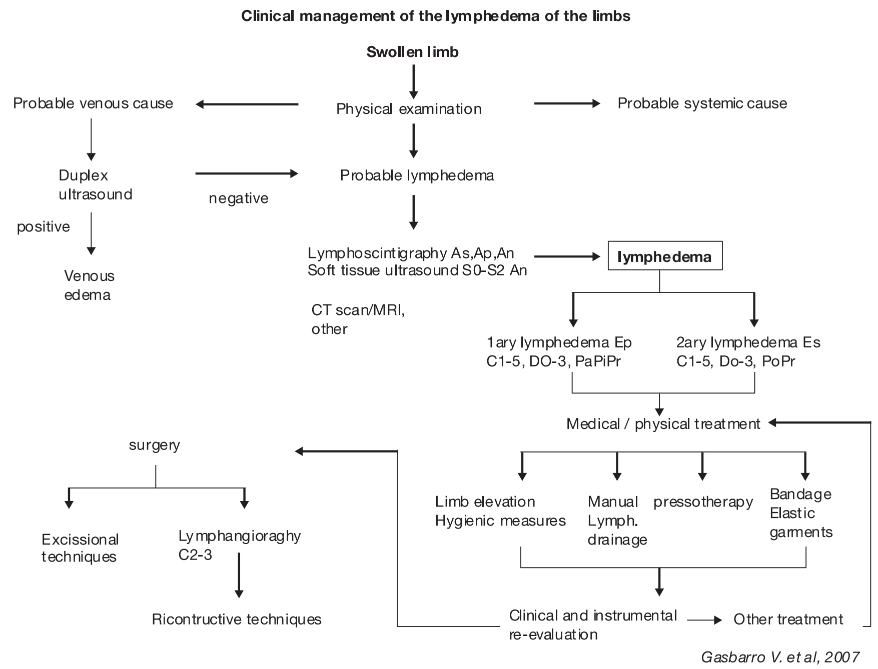

Table 2

All patients were then selected by C CEAP-L class (C stage of disease [1-5], localization and involvement grading) (Tables 3, 4, 5).

Table 3 - Clinical classification

C1	No edema (pre clinical stage)	1 point
C2	Edema that disappears with night rest	2 points
C3	Edema that persists with night rest	3 points
C4	Fibrotic edema	4 points
C5	Elephantiasis with skin lesions	5 points

Table 4

Lower Limb (LL)	Upper Limb (UL)
FOOT (1 point)	HAND (1 point)
LEG (1 point)	FOREARM (1 point)
THIGH (1 point)	ARM (1 point)
GENITAL (1 point)	SHOULDER (1 point)
TRUNK (1 point)	

Table 5

Group 1 (Diamagnetotherapy + Compression)	Group 2 (Compression)
C2 3 limbs	3 limbs
C3 15 limbs	14 limbs
C4 3 limbs	4 limbs

In a second step the patients, recruited according their class, were randomized to get homogeneous samples for a more reliable final evaluation. The echography was performed using a 7.5-10 MHz probe with Kontron Sigma and Philips 7.5-10 MHz devices. The exploited parameters were the subcutaneous tissue thickness, the presence of hyperechogenicity in the sub-cutis (signifying the presence of free lymph, that is, “lymphatic holes”) [24]. We considered the interstitial trabecular meshworks that at this stage appear thickened, hyperechogenic and fragmented. Using a limb echographic mapping we observed high fibrosis and lymphatic accumulation areas; the same issues were evaluated after the therapy.
The application of diamagnetic therapy was performed according to the scheme:
Operative way: Liquid shift; Intracellular 20%; Extracellular 100%.
Diathermia with resistive system, electrical resistance of 500-1000 Ohm according to the measured impedance (the device is provided with an impedance detector that permits to highlight tissue areas with high resistance to magnetic fields, where it is necessary to increase the electrical resistance up to 1000 Ohm).

Executive procedure: The massage was performed following the lymphatic draining directions, in this way combining the advantage of the hand-made lymphatic drainage with the energy developed by the machinery [25].
The duration of diathermia application was 30-40 minutes and it was repeated three times per week for about two months (for a total of 20 applications).
The study was six months long in order to evaluate possible in-time negative effects of this therapeutic methodology.

Side effects: 4 patients (10% of the sample) showed, in the early stage of the therapy, a temporary warm sensation, nausea and urgent diuresis stimulus.

RESULTS

Clinical (limb’s circumferences and consistency) and instrumental (soft tissues echography) controls showed a positive result in patients treated both by diathermia and compression with respect to those treated only by compression.

Clinical evaluation:

The clinical improvements, revealed by using the very reliable CEAP-L classification, are summarized in the following table 6.

Table 6

	Group 1 (T0)	Group 1 (T1*)	Group 2 (T0)	Group 2 (T1*)
C2	3	6	3	3
C3	15	15	14	15
C4	3	0	4	3

These results deserve an in-depth analysis. Indeed, even if they could appear of an ordinary level, they actually are not. In the group 1 (diathermia + compression) we obtained in the totality (3 limbs, 100%) of class C4 limbs a regression of trophic troubles like lymphatic ulcers. As a consequence of this improvement in the disease, they were declassed to a C3 class. This result is, in our opinion, of great clinical significance. The same happened to the three patients (20%) that moved from C3 class to the C2 one. On the other hand, by using only the compression therapy the clinical outcomes were basically irrelevant.
To make the results more meaningful we also used a clinical gravity score, that led to the following data (Tables 7, 8):

Table 7

CLINICAL GRAVITY SCORE
1 point for each area of the limb involved
1 point for each limb involved
2 points for other areas involved (genitals, shoulders)
1-4 points according to the stage of edema
1 point symptomatic edema
1-3 points according to the stage of disability

Table 8

Clinical gravity score	Group 1 (T0)	Group 1 (T1*)	Group 2 (T0)	Group 2 (T1*)
5	5	8	3	3
6	11	11	12	13
7	0	1	2	1
8	2	1	0	1
9	3	0	4	3

Instrumental evaluations:

Together with a decrease in the oedema volume, patients treated by diathermia + compression (Group 1) showed a meaningful decrease of tissue's consistency (hard oedema becoming soft oedema). In details, the echographis evaluations pointed out an improvement in the echographic derma and hypoderma structure, with more homogeneous and thinner connective shoots, an hypo-anechogenic appearance of the superficial loose cellular tissue, a reduction of the connection synechiae between derma and hypoderma and between hypoderma and superficial muscular fascia, with a consequent better excursion of the muscular district. In addition, at the end of each treatment cycle we observed a reduction of the lymphatic holes and lakes [26], with a consequent decrease of the circumference measures [cB (ankle) = –3 cm; cD (knee) = –4 cm; cG (thigh root) = –6 cm] (Fig. 2) e [cC (wrist) = –2 cm; cE (elbow) = –3,5 cm; cG (arm) = –5 cm] (Fig. 3) and most of all the transformation of a hard oedema in a soft one. In this way, the limb became more compressible (decrease in the tissue stiffness) leading to a better response to the compression therapy [27].

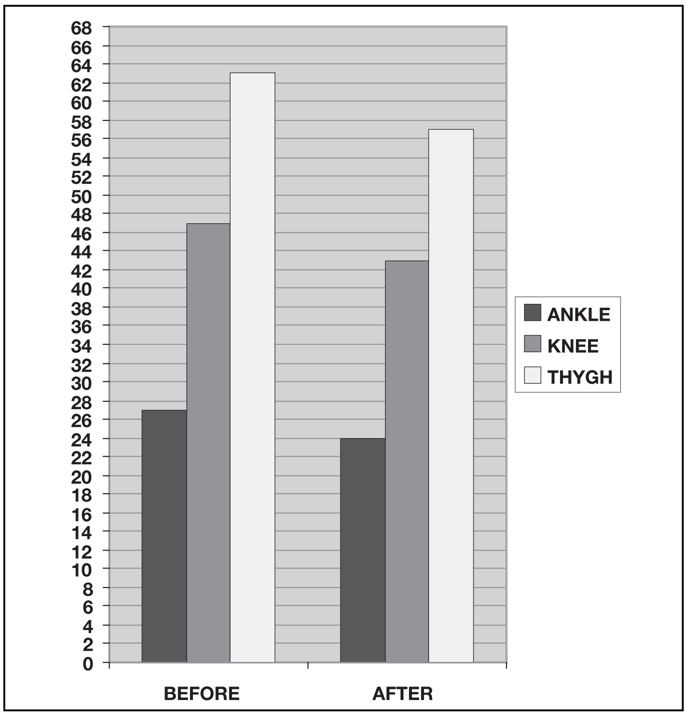

Fig. 2 - Average decrease in the inferior limb's circumferences measures.

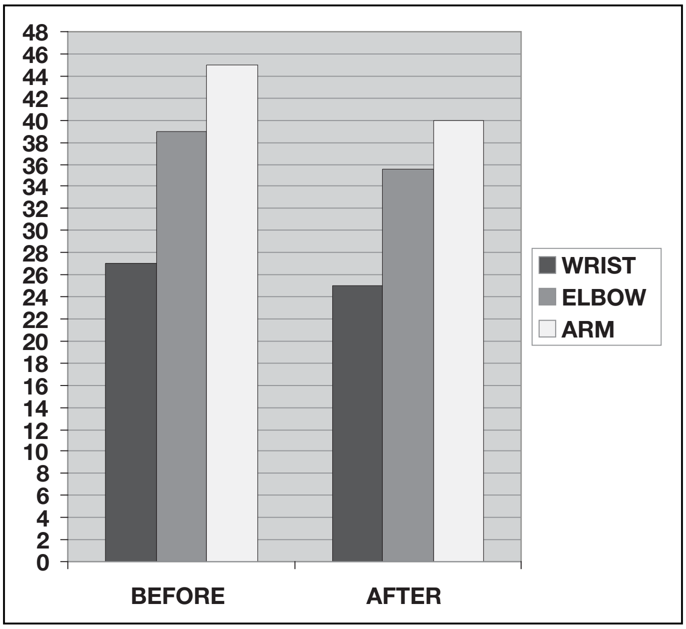

Fig. 3 - Average decrease in the superior limb's circumferences measures.

CONCLUSIONS

The data we obtained confirm the validity of the therapeutic approach (CTU Mega 18), even it is certainly integrable with all other therapeutic treatments of oedema, in general, and of lymphoedema, in particular [28]. It has shown its efficiency thanks to the different and synergic actions (diamagnetic force acting on the water, thermal effect and possible pharmacologic subcutaneous transport), that led to good results, both on clinical and on instrumental side. In particular, in Group 1 we observed a clear clinical improvement with respect to Group 2, testified by the CEAP-L classification and by the clinical gravity score, as well as an instrumental improvement pointed out by echographic images [29]. The safety [30] of the technique, moreover, has been benchmarked by the absolute irrelevance of registered side effects. In conclusion, the patients satisfaction and the objective improvement in both clinical and instrumental data, together with its simplicity, make the proposed technique, possibly integrated with other approaches, a new fundamental tool in the therapy of lymphoedema.

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VOLUME 21 • No. 61 • 2010
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CHARACTERISATION OF LYMPHATIC VESSELS IN NON SMALL CELL LUNG CARCINOMA AND CORRELATION WITH LYMPH NODE METASTASIS: A PRELIMINARY MORPHOLOGICAL STUDY

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ABSTRACT

The lymphatic system is an important route of metastatic dissemination in carcinoma. However tumour associated lymphatic vessels are poorly characterized compared to blood vessels. Lymphatic vessel density (LVD) and lymphatic vessel invasion (LVI) have been investigated in some studies of NSCLC, creating sometimes contradictory data.
We performed a preliminary study of 15 surgically removed NSCLC to test the feasibility of LVD quantification using a recent commercially available antibody (D2-40) and analyse the relationship between LVI and lymph node status. We pointed out the heterogeneity of lymphatic vessel distribution and the special need of standardization in the quantification step. Our work suggests that LVI is a relevant parameter to correlate with lymph node status but more validation and quantification work is needed.

KEY WORDS: lung cancer, metastasis, lymphangiogenesis.

INTRODUCTION

Metastatic tumour spread is the leading cause of tumour mortality. The lymphatic system is an important route of metastatic dissemination in carcinoma [1, 2, 3]. Thus lymph node metastasis is a major adverse prognostic factor and a key item of the UICC International Union Against Cancer TNM classification of malignant tumours. However tumour associated lymphatic vessels are poorly characterized compared to blood vessels. Indeed the essential role of angiogenesis (new blood microvessel formation from pre-existing ones) in tumour development is now recognized, whereas the existence and role of lymphangiogenesis are still under investigation [2, 3, 4]. However, resulting from lymphangiogenesis or co-option of pre-existing vessel, lymphatic vessels are present in non small cell lung carcinoma (NSCLC), and there is a need for characterisation of the lymphatic metastatic spread. Lymphatic vessel density (LVD) and lymphatic vessel invasion (LVI) have been investigated in some studies of NSCLC, creating sometimes contradictory data [5-9]. New tools of lymphatic staining have emerged [10]. We performed a preliminary study of 15 surgically removed NSCLCC to test the feasibility of LVD quantification using a recent commercially available antibody (D2-40) and analyse the relationship between LVI and lymph node status.

METHODS

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissues from 15 randomly selected NSCLC were retrieved from the archives of the Department of Pathology (Pompidou Hospital). The patients were treated with lung wedge resection, lobectomy or pneumonectomy with lymph node dissection.
Tumour slides were processed for immunohistochemistry as follows: After deparaffinization, microwave antigen retrieving was done in citrate buffer pH6. Anti anti–D2-40 mouse monoclonal antibody (clone D2-40, diluted 1:200; Dako, Carpinteria, CA) was diluted to 1/200. An indirect immunoperoxydase technique was done on Ventana NexES staining module (Ventana Medical Systems, Tucson, AZ).

Lymphatic vessel density (LVD)

Lymphatic vessels were highlighted by D2-40 immunostaining. Lymphatic vessel density was assessed without knowledge of patient's lymph node status. Lymphatic vessel density was counted as described : sections were scanned at low power (x4obj) to identify areas with the greatest number of lymph vessel (hot spots). The LVD count was expressed as the total number of lymphatics identified in ten successive x20obj (intermediate power) fields within the hotspot. Lymphatic vessel density was

Table 1 - Characteristics of tumours. pN status: according to the pathological TNM classification of malignant tumours; Ca: carcinoma; LC: large cell; LVD: lymphovascular density; LVI: lymphovascular invasion

Case	Histological type	Tumour size (cm)	pN status	LVD	LVI
1	Adenosquamous Ca	2,5	N0	75	Yes
2	Squamous Cell Ca	3,1	N0	4	No
3	Sarcomatoid Ca	8	N1 cont	5	No
4	Adenocarcinoma	8	N1 cont	10	No
5	Squamous Cell Ca	2,5	N0	15	No
6	Adenocarcinoma	3,5	N0	79	Yes
7	Adenocarcinoma	2,9	N1	26	Yes
8	LC undifferentiated Ca	2,4	N0	36	Yes
9	Adenocarcinoma	3,5	N0	71	Yes
10	Adenocarcinoma	1	N2	23	Yes
11	Adenocarcinoma	1,7	N1	74	Yes
12	LC neuroendocrine Ca	1,2	N0	49	Yes
13	Adenocarcinoma	2,3	N0	24	No
14	Adenosquamous Ca	2,5	N0	54	Yes
15	Adenocarcinoma	2,5	N0	14	No

correlated with lymph node status at the time of primary tumour resection. Tumours n° 3 and 4 invaded one proximal lymph node by contiguity and were included for analysis in the N– group.

Lymphovascular invasion (LVI)

LVI was defined as the presence of tumour cells in at least one lumen of a D2-40 stained vessel, inside or in the immediate vicinity of the tumour.

RESULTS

Tumour characteristics

There were 8/15 adenocarcinomas, 2/15 squamous cell carcinomas, 2/15 adenosquamous carcinomas and 3 other large cell carcinomas. Average size was 3.2 cm (range 1-8 cm). 10 cases were classified pN0, 2 cases were pN1 by contiguity, 2 cases were pN1 (hilar nodes) and 1 was N2 (mediastinal node). For further analysis pN0 and N1 by contiguity (not involved by lymphatic route) were reported as N–, and distant pN1 or pN2 as N+.
Pattern of lymphatic vessel staining
The intratumoral distribution of LV was highly heterogenous in 13 out of 15 tumours: most of the tumour surface was devoid of LV, as illustrated in figure 1. All tumours contained at least one peripheral LV positive area whereas only 6 tumours out of 15 displayed a central LV positive area. The LVD was also very variable between tumours. Mean LVD was 37.3 ± 27.4 LV per 10 IPF (range 4-79). LVD was not different between N– and N+ tumours (Figure 1A).

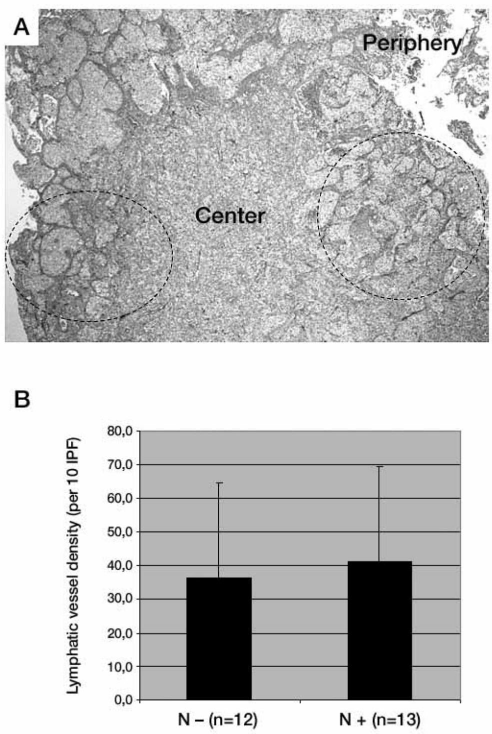

Fig. 1 - A: Case n° 11 stained with anti D2-40, illustrating the peripheral hot spots (circles) and heterogenous density of lymphatic vessels. Obj x 4.
B: Comparison of LVD between N– and N+ cases.

Characterisation of lymphovascular invasion

Lymphovascular invasion as evidenced by tumour embolism was frequent, occurring in 9 tumours out of 15 (60%) (Figure 2A). LVI was more frequent when LVD was above the average of 37.3 (100% versus 33%). Accordingly LVD was higher in LVI tumours (Figure 2B). 33.3% of LVI+ were N+, whereas there was no N+ in LVI – cases (Figure 2C).

DISCUSSION

The recent identification of lymphatic markers that can accurately discriminate lymphatic vessels from blood vessels in tissue sections has enabled studies on the lymphatic system, especially in tumours.
A comparative study of antibodies directed at LYVE-1, Prox-1 and the D2–40 antibody on serial sections of breast carcinomas indicated that significantly more intratumourous lymphatic vessels stained with D2–40 , thus demonstrating that this marker is highly sensitive for lymphatic endothelium [10]. The commercially available monoclonal D2–40 antibody specifically recognises human podoplanin a 38-kd surface glycoprotein. According to the first conference of consensus we performed lymphatic vessel staining with D2-40 antibody [10].
In our small series, LVD was not higher in N+ patients than in N–. This has also been reported by Faoro *et al.* [6] in a series of 78 NSCLC. In the study of 103 NSCLC by Renyi-Vamos *et al.* [9], it was determined that LVD at the tumour periphery was significantly higher in lymph node metastatic tumours. None of the 3 studies used the same method of quantification for lymphatic vessels. Notably we counted all vessels in 10 x20obj fields, Faoro *et al.* [6] determined a maximal LVD by x20 field, and Renyi-Vamos *et al.* [9] used a morphometric analysis to quantify a mean LVD per square millimeter. This underlines a general concern about the quantitative studies of both blood and lymphatic vasculature: the heterogeneity of methods.
Recent consensus conferences have tried to establish recommandations to enable comparison of the data. However work on lymphatic vessel density/lymphangiogenesis is still emerging and needs validation [4, 10].
The recent discovery of VEGFR-3, mainly expressed on the lymphatic endothelium and of its secreted ligands VEGF-C and VEGF-D has enabled the study of lymphangiogenesis, i-e the formation of new lymphatic vessels from pre-existing ones. Indeed the VEGF-C/VEGF-D/VEGFR-3 axis constitutes the signal transduction system for lymphatic endothelial cell growth, migration, and survival [2, 3]. Animal experiments demonstrate that tumour-induced lymphangiogenesis promotes metastasis to lymph nodes and beyond. However the occurrence of lymphangiogenesis in human tumours is still a matter of controversy.
The lack of a tight correlation, generally reported as in our series, between LVD and lymph node metastasis suggests that lymphangiogenesis is not an absolute prerequisite for metastasis to lymph nodes [2]. If the tumour is located in an area with a high lymphatic vessels density, tumours could for example co-opt these vessels and efficient entry of tumour cells may take place in the absence of lymphangiogenesis.
Accordingly, we observed that LVD was very low compared to blood vessel density (data not shown) and extremely heterogenous with large zones devoid of lymphatics. Given that lymphatic vessels exist in lung bronchus and parenchyma this could also reflect vessel cooption rather than lymphangiogenesis. As suggested by the consensus conference, double staining with D2-40 and the proliferation marker Ki67 should be done to investigate the presence of proliferating lymphatic endothelial cells, reflecting lymphangiogenesis. Some authors have divided NSCLC into angiogenic and non-angiogenic tumours depending on the vascular pattern [11]. This may also be the case with lymphangiogenesis but specific patterns have to be defined.
Existence of lymphovascular invasion is a mandatory item of pathological report for some tumours like breast and colon carcinomas, whereas it is not in NSCLC since it is not a validated prognosis indicator. Should it become of interest to systematically assess lymphovascular invasion in NSCLC, D2-40 staining would improve the sensibility of detection compared to HES, as reported in many other tumours [12, 13]. In this study of 15 cases we found as much as 60% of LVI + with the help of D2-40. The majority of LVI was not reported in the pathology form. Lymphovascular invasion evidenced by IHC does not warrant lymph node metastasis but N+ cases were all LVI+ and there was no N+ in LVI- patients. It may be useful to quantify LVI in further studies to try to establish a correlation with lymph node invasion [8].
Moreover, since methods are developed to assess micrometastasis in lymph nodes, LVI could be more correlated to the occurrence of

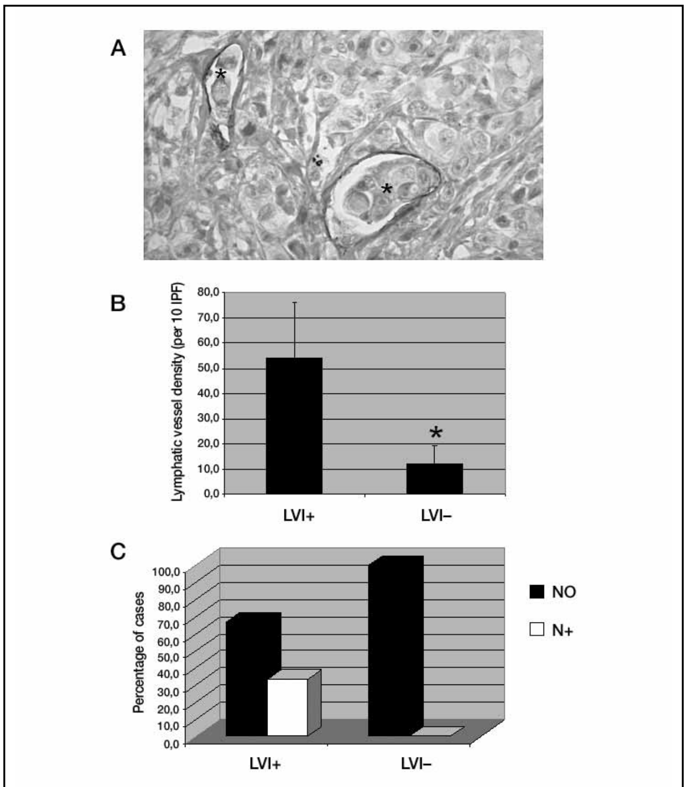

**Fig. 2 - A: Lymphovascular invasion as evidenced by tumour cell embolism (stars) inside D2-40 positive vessels.
B: Comparison of LVD between LVI+ and LVI– cases.
C: Comparison of N status in LVI+ and LVI– cases.**

micrometastasis than macrometastasis [14]. This hypothesis is currently under investigation. In conclusion, this preliminary study on a series of NSCLCC confirmed the feasibility of lymph vessel staining by D2-40 and quantification, pointed out the heterogeneity of lymphatic vessel distribution and the special need of standardization in the quantification step. Our work suggests that LVI is a relevant parameter to correlate with lymph node status but more validation and quantification work is needed.

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SUBSTANCE P IN LYMPH-EDEMA AND ITS CHANGE AFTER PHYSIOTHERAPY

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ABSTRACT

Introduction: The mechanism how the physiotherapy by magnetic fields, vibration and hyperthermia is effective in the treatment of lymphedema, is not known. After some treatments of lymphedema the contralateral untreated side of the extremity is reduced in the volume. No body knows how. To answer these questions this investigation has been performed.

Material and method: Substance P is examined in five cases of secondary lymphedema of the lower extremity before and after the physiotherapy by magnetic fields, vibration and hyperthermia.

Result: Substance P becomes elevated after the physiotherapy.

Discussion: All four subtypes of vanilloid receptors are considered to have been stimulated. The excreted substance may strengthen the contraction of the lymphatic smooth muscle making this physiotherapy more effective. It may explain why the untreated contralateral side of the extremity becomes decreased in volume after the treatment.

Conclusion: Substance P becomes increased after physiotherapy of lymphedema by magnetic fields, vibration and hyperthermia.

INTRODUCTION

Capsaicin, a component of red pepper is known to stimulate vanilloid receptors(TRPV1) releasing neurotransmitting substance such as substance P and calcitonin gene-related peptide (CGRP). These substances are considered to stimulate the smooth muscle cell of the lymphatic wall. If these substances are excreted after the physiotherapy by magnetic fields, vibration and hyperthermia [(1)], an excellent result of the lymphedema treatment by this physiotherapy is well explained. Piller et al has demonstrated untreated side of the extremity becomes reduced in volume when the lymphedematous extremity is treated by transdermal stimulator [(2)]. No body knows how. However this neurotransmitting substance may explain it. Hasegawa and Ohkuma have already reported an increased CGRP in lymphedema after the physiotherapy [(3)].

MATERIAL AND METHODS

Plasma is taken from 5 cases of secondary lymphedema respectively before and immediately (within 5 minutes) after the physiotherapy by pulse magnetic fields, vibration and hyperthermia [(1)]. The plasma is evaluated for substance P by enzyme immunoassay Kit (Catalog No. 900-018, Assay Design, Inc.). All patients become free from edema after the treatment with the circumference difference (10 cm above or beneath the patella edge) less than 1.0 cm between the lymphedematous and uninvolved contralateral extremity in case of unilateral lymphedema and with a subjective evaluation by the patients themselves in bilateral lymphedema.

RESULTS

The result is listed in the Table 1. The substance P is increased after the treatment (Fig. 1).

Table 1 - Result of Enzyme Immunoassay for Plasma Substance P in Lymphedema before and after the Physiotherapy by Magnetic Fields, Vibration and Hyperthermia [(1)]

	Before treatment (pg/ml)	After treatment (pg/ml)
Substance P	130	173
	178	191
	165	240
	295	305
	345	508

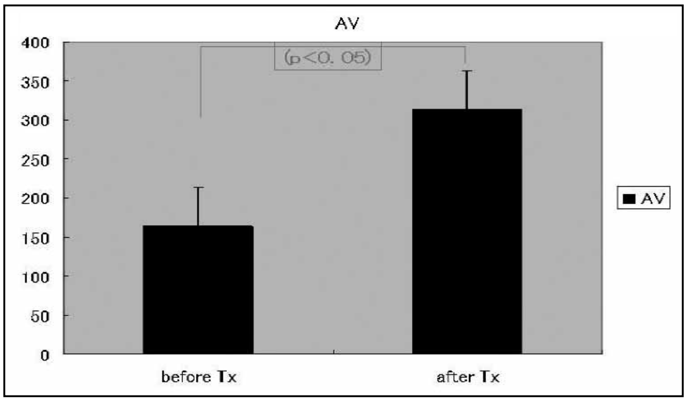

Fig. 1 - Column demonstration of the plasma value of substance P taken from 5 cases of secondary lymphedema before and after the physiology by magnetic fields, vibration and hyperthermia. The value becomes increased after the treatment ($P < 0.05$).

DISCUSSION

This increased substance P can be excreted from the nerve after the physiotherapy and stimulate contraction of the lymphatic smooth muscle inducing more lymphatic transport of the edema fluid (Fig. 2). This physiotherapy may stimulate all 4 subtypes (TRPV1 to TRPV4) of vanilloid receptors (Table 2). That is why this physiotherapy treatment is more effective than manual massage or pneumatic compression which stimulates only TRPV2 and TRPV4 (Table 2). A few patients complain of headache during this physiotherapy [1] which may attribute to his increased substance P. If nitrous oxide is produced during this physiotherapy, cGMP becomes elevated releasing substance P or CGRP. Whether the magnetic fields put influence on vanilloid receptors or not must be investigated in future.

Table 2 - Subtype of Vanilloid Receptors and its Stimulation & Localization (the underlined: those which are associated with this physiotherapy).

Stimulated by	Localization
TRPV1 temperature <43 ºC, capsaicin, acid inflammatory chemical mediators, and HO-β sanshool	nerve, skin, urinary bladder smooth muscle cell, vascular smooth muscle cell
TRPV2 temperature >52 ºC, mechanical	nerve, lymphatic smooth muscle cell (rat)
TRPV3 temperature >35 ºC 2-AEDB*, naphthalene	nerve, keratinocyte
TRPV4 temperature >35ºC, mechanical	keratinocyte, lymphatic smooth muscle cell (rat)
TRPV5	
TRPV6	skin
TRPM1	
TRPM2	skin
TRPM8/CMR1 menthol, cold stimuli (<25°C)	nerve
TRPAI cold stimuli (<17°C), cinammaldehyde (horse raddish, garlic, cinamon)	nerve

(*) 2-aminoethoxydiphenyl borate.

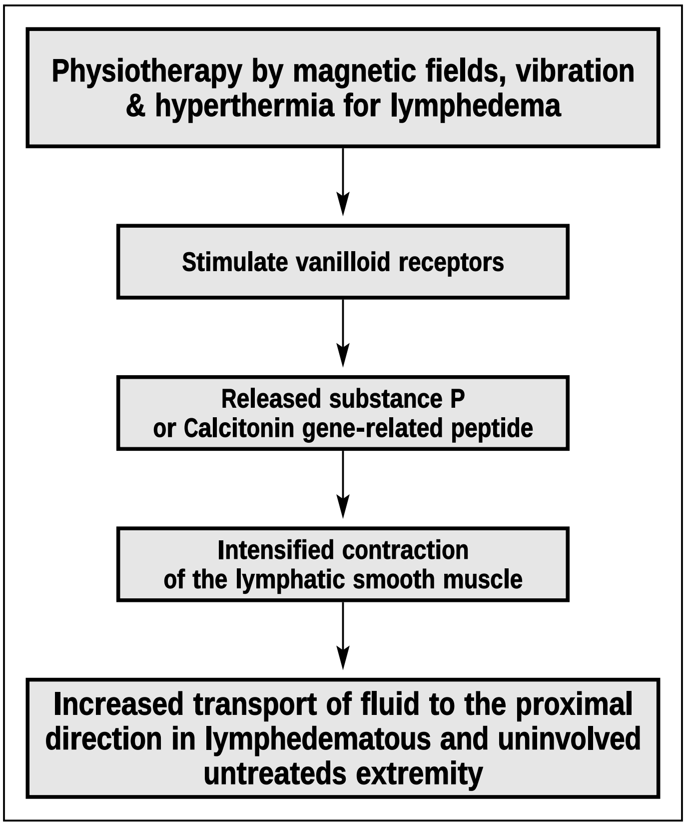

Fig. 2 - Possible mechanism how physiology by magnetic fields, vibration and hyperthermia intensifies contraction of the lymphatic smooth muscle cell.

CONCLUSION

Substance P is increased in lymphedema after physiotherapy by magnetic fields, vibration and hyperthermia. This may explain why this physiotherapy is so effective intensifying the contraction of the lymphatic smooth muscle cell. It explains also volume decrease of the untreated contra-lateral extremity in lymphedema after the treatment.

ACKNOWLEDGEMENT

This papers were presented in the 22nd International Congress of Lymphology, Sidney, Australia 2009.

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VOLUME 21 • No. 61 • 2010
INDEXED IN EXCERPTA MEDICA

LYMPHOEDEMA: WHAT CAN BE MEASURED AND HOW… OVERVIEW

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1. INTRODUCTION

The evolution of lymphoedema during physical treatment appears quite easy to measure, especially if we limit ourselves to considering the decrease in its volume, and furthermore if we limit the volume's measurement to the geometry of the arms, forearms, legs and thighs to a cylinder (lymphoedema reside mostly in these anatomical segments). On the other hand if we want to observe more complex anatomical areas such as the breast or scrotum, and especially when we take into account other parameters that are equally as interesting, such as the quality of the skin, it's water density, the thickness of the dermis etc., the task of remaining precise becomes much more complex.
With regard to the measurement of the shape of the oedema, a series of pictures taken in standardized conditions (which are explained in detail below), can suffice for the analysis of the treatment's results and the control of its efficiency. But how can we deal with more complicated geometries like the root of the limb, hands, feet, the face, breasts, external genitalia…and again other parameters that are less visible to the naked eye such as the thickness of the dermis, the variation of the subcutaneous tissue, the skin's temperature, the quality of the skin, the tonicity of the oedema, the joint's function, the quality of life, etc. All of them are measurable and important in the evaluation of the treatment's efficacy. Regular measuring in the daily practice represents an important part in the assessment of the oedema's evolution, and the more parameters the therapist can avail of, the better equipped they will be to adapt the treatment to each patient's specificities.
Another interesting application of the knowledge that can be obtained from measuring the affected area is the predictive factor or early indicator: detecting slight changes that are invisible to the naked eye, and thus availing of this information to commence treatment before the possible onset of a clinically significant lymphoedema.
Our aim is to discuss the most common parameters that are available for measurement, and the methods used to measure them, and to weigh their advantages and disadvantages to facilitate the comparison of different treatments and methods both on a theoretical level and in a practical setting.

2. MEASURE OF VOLUME AND PERIMETERS

The first impression you get when you observe a part of the body affected by lymphoedema, is the deformation of that area, either in comparison with the other side, or compared to what can be considered as "normal".
Thus it seems logical to try to measure the variation in volume of the oedema, firstly in order to monitor the treatment and secondly to motivate the patient by providing quantifiable evidence of improvement throughout its evolution.
There are two different approaches for measuring volume: a direct and an indirect technique.

1.1. The direct technique consists of a derivation of the Archimedes's Principle: a body immersed wholly or partially in fluid (liquid or gas) displaces a quantity of liquid equal to its immersed volume. Consequently the oedematous segment will be immersed into a recipient filled with water and the water's displacement will be gauged. If you examine more closely the quality of this method, you notice that there is a non negligible potential of errors[1]. The main reasons rely on the superficial tension, the adsorption phenomenon, the reproducibility of reference points on the wrist, the microscopic structure of the skin's surface, the temperature of the water, the texture of the recipient's inner surface, the proportion between the size of the immersed object and the water volume and finally the error on the lecture of the values.

Therefore many authors proposed or experimented various types of measuring systems in the field of volumetry by immersion. Some proposed to fix a pressure transducer in the bottom of a container, others measure the rate of the water displacement and still others recuperate the quantity of displaced water in a graduated beaker in order to weigh or measure it. Ph. Lefèvre[2] suggested a very sensitive method in which he set up a volumetric device which allows the measurement of the hand with an accuracy of 1%, for volumes between 250 and 300ml. In this method a metallic needle is placed on top of the water surface in order to measure the variation of the electric resistance due to changes in the height of the water.
The majority of the apparatus available today, even in the best of conditions result in a 3% error, it is for this reason that we cannot refer to the immersion technique as the golden standard, as it has sometimes been considered by other authors. *"Measurements of volume can be misleading, particularly when the swelling of an obviously oedematous limb is restricted by tissue resection or contraction from fibrosis. Furthermore, volume measurements are prone to error because all oedema varies widely according to the time of day, the stage of the menstrual cycle and physical activity"*[3].

Literature overview of this topic shows us that the immersion method is an inappropriate method in the majority of situations, except due to their complex geometry, when dealing with the particular case of an oedema of the hand or foot. In practice when dealing with the limbs, this procedure requires a whole collection of different containers and apparatus that renders it unpractical, messy and time consuming. Furthermore with regards to hygiene, since people with lymphoedema often have concomitant skin alterations, the appropriateness of this procedure is questionable and it is also not suitable for patients in the immediate postoperative period.

Another weakness of the water displacement method is that the root of the segment is not immersible. Knowing the variation within this area is important to understand the displacement of the oedema as it plays a key role in determining whether the oedema is leaving the limb. Finally the water displacement gives only a global view of the reduction in the oedema, and not a precise segmental analysis which is required for monitoring appropriately multi layer bandaging.

1.2. The indirect technique comprises the acquisition of the perimeters' values that are then integrated into geometrical formula. There are two possibilities, the simplest method is to consider the lymphoedema as the addition of little cylinders that are 4cm long. In this case we can apply the simplified formula:

$$V_{limb} = \frac{\Sigma Z^2}{\Pi}$$

In which Z is equal to the circumference and where it is of utmost importance to take the measures every 4cm precisely.
This simplified method induces a substantial error because of the fact that a lymphoedema is unlike a tube in that it is not uniform and produces skin folds.

The other calculating method, using the truncated cone's volume tries to reduce this error by taking into account two successive circumferences. In this case the distance between two circumferences is non defined but the closer the measurements of the circumferences the more accurate the resulting volume.

$$V_{limb} = \frac{\Sigma x^2 + y^2 + xy}{3\Pi}$$

In this equation x equals one circumference and y the other.

Authors have tested the reliability between these two methods and the water displacement volumetry and they have concluded that the circumferential measurement has a better reliability. Due to the fact that interrater reliability is quite low, but that the intrarater reliability is higher, ideally a patient should always be evaluated by the same therapist[4].
These measurements will vary according to the reproducibility of the reference marks, the moment of the day or the activity before the measurement. In a bilateral lymphooedema, the comparison between the two limbs remains interesting to evaluate if one limb reacts better than the contralateral limb after the same treatment.
The tape measurement technique has the strength that it allows the analysis of segments individually and this, up until the root of the limb.

In the situation where hands or feet cannot be immersed in water there exists another technique in which the measuring tape is wrapped around the hand forming an '8', authors[5] have shown that this technique is an ICC of 0.9 compared to the immersion technique.

1.2.1. Other technology allows us to obtain values for the circumference of the limb, such as optoelectronic systems based on infrared diodes or LASER.

- Optoelectronic volometer (Fischbach & Goltners - 1986) is a system based on infrared diodes which record the shadow of the limb. This system has a good accuracy if the geometry of the limb is regular, however if this is not the case it can generate an inherent error of 10-15% when dealing with the complex geometry [6] of an arm affected by lymphoedema with a lot of skinfolds. Furthermore these systems require a large abduction to permit the machine to access the isolated limb especially at the root and due to the configuration of the machine the extremity and the root of the limb end up being neglected. For example in the case of the leg, the foot is held in place by a saddle that blocks the light and hence the measurement. These systems are very expensive for a private practice and therefore are realistically attainable only in much bigger organisations.

- LASER technology is a promising technique that can also be used in measuring the evolution of lymphoedema. These techniques have been used for a long time when dealing with lumbar problems, casts and corset fabrication. When applied to lymphoedematous limbs it will allow a fast, precise and reproducible perimetry. It has passed its phase of development, but remains expensive and is in hand of industry.

3. MEASUREMENT OF THE SKIN FOLDS

The components of a lymphatic oedema, depending on the conditions and different elements considered, can leave the affected area by three different evacuation routes; venous, lymphatic and interstitial. Intermittent compression therapy and multilayered bandaging applied to the limb cover the whole oedema up until the root of the limb, at this level part of the oedema is therefore pushed closer to the root of the limb and to the chest or hip area directly in relation with the limb. The trained hands of a physiotherapist can aid these fluids in rejoining lymphatic substitution pathways that drain into the contra-lateral lymphnodes, or simply attaining sub cutanous region where physiological drainage continues to run well. These sub cutanous routes are filled with interstitial fluid, and for this reason skin folds in this region are much thicker. The measurement and comparison of these skin folds with a caliper, compared to the contra-lateral side, enable us to establish a clear picture of which regions are receiving the liquid coming from the affected limb. This tool is therefore very useful for the management and treatment of lymphoedema.
Another advantage of this utensil is its use when we have to deal with “flatter” areas of the body like the chest, hips or breasts. A caliper is much better adapted to these forms, and presents the ideal solution for evaluating lymphoedema through the measure of skin folds in this area with an acceptable reliability and accuracy.

4. IMAGERY BY PHOTOGRAPHY

An easy, quick, and cheap method in the assessment of the evolution of a lymphoedema through out treatment remains photography. Taking pictures helps patients on the one hand to visualize the reduction of their swelling, and can act as a form of psychological support especially when the treatment is heavy and the oedema persistent, on the other hand it’s a precious piece of evidence for communication with other therapists. In both cases, they only have to throw a glance at the pictures to acknowledge the relevance of the treatment.
The relative simplicity of photography also represents its weakness: small modifications of the volume are not visible, the picture is only two-dimensional, the control of luminosity and the difficulty of placing patients in the same conditions for every picture pose a problem.
Thus, the use of photography helps the therapist to demonstrate with ease the treatment’s benefit in the case of a big lymphoedema, where the volume reduces quickly, but for smaller oedema or slower evolutions, because of the difficulty of taking measures from the pictures, it is not exhaustive. Accordingly, photography constitutes thus first and foremost a qualitative and not a quantitative tool.

In order to obtain a reproducible photograph where we can assure that the patient is in the exact same position, we propose a simple method based on the grid function that is available on all modern cameras.
This grid is made up of 9 rectangles, each composed of a ratio between their width and length. This ratio is based on the Golden Ratio and Fibonacci Numbers [7].
Our idea is to magnify these rectangles and draw then on the wall, or background, in front of which the photograph can be taken every time. We therefore enable a referential that can be reproduced with ease, and that is reliable, so when taking the photograph the lines can be aligned and thus the exact same frame is used.

5. IMAGERY BY DEEP INFRA-RED THERMOGRAM

The backflow of lymph into the superficial tissues, also called « dermal backflow », is a pathognomic sign of lymphatic insufficiency. The backflow is due to the incapacity of lymphatic collectors of one area to drain the lymph properly. The lymph recedes into the initial lymphatic network inside the dermis, where liquids and macromolecules cannot be reabsorbed. Because of local pressure, they have to find their way through the dermis to another area of the body, for example up to the root of the limb until they find areas where the physiological drainage is efficient. The dermal area that is not sufficiently drained firstly increases in pressure, and then becomes more voluminous. This lymphatic stasis increases the pressure on local tissues causing among other things a compression of the arteriovenous capillaries.
From a clinical point of view, in the first stage this physiopathological state occasions a paleness and coldness of the skin. This cold area can be identified and measured precisely with a deep infra red camera, that therefore pin points the areas that are affected by dermal backflow, directing the clinician to this site that demands specific attention in the global treatment of lymphoedema.
Despite the fact that lymphoscintigraphy represents the golden standard examination for the diagnosis of the dermal backflow, because it requires an injection of radiocolloids, it is not conceivable in a regular follow-up of a treatment. This leaves a greater place for thermograms in treating lymphoedema.

Following this first stage of coldness if the stasis is important and makes the tissues suffer, the skin becomes warmer, because of the beginning of the inflammation process. Deep infra red thermograms enable us to make an early detection, because of the sensitivity of the camera and are a valuable tool in this situation. The use of infra red thermal cameras enables us to obtain surface thermograms of high precision and excellent resolution for the diagnosis and evaluation of the treatment's efficiency with lymphoedema accompanied by dermal backflow. Thanks to their easy mode of operation and quick manipulation, these cameras can be used in a daily routine. We have completed preliminary studies with promising results, but further investigation is needed on this topic.

6. TONOMETRY

Tissue tonometry is the measurement of mechanical change due to external pressure that displaces interstitial fluid to leave a depression or pit in an oedema. Tonometry registers the depth of tissue compression by a known mass over a fixed time interval. It can be an important tool to mesure the success of a lymphoedema treatment and management program, although rarely used. The measurement of the depth of pitting in lymphoedema has been described using a tonometer by various authors [8].

The Tissue Tonometer appears to be an easy-to-use, fast and non-invasive method available to assess the characteristics of the swollen limb. Its accuracy and reproducibility in assessing pitting oedema in oedematous and lymphoedematous tissues were confirmed by a number of authors [9]. The Tonometer can be set at different weight levels of 70, 140 and 210 [10] grams that gently push a plunger onto the skin.
The reliability between tonometry and water displacement or circumference measurements seems not to be effective [11].
The interpretation of results obtained through tonometry need to be closely scrutinized. Firstly they should be analyzed with relation to time, secondly it is very important to correlate the results with histological evidence. Often we are too quick to conclude that a non pitting oedema is fibrotic and therefore non reversible. Through clinical experience we know that these oedemas are more resistant to the pitting test as observed with the tonometer, but also we know that with intensive physical treatment and adapted technique we can obtain substantial improvements. Authors consider that the more an oedema is "tonic" the more fibrotic it is, they asses "fibrotic indurations" by tonometry [12]. We are not convinced by these conclusions, because there exist non pitting oedemas, that are resistant to a tonometer, but that react very well and quickly under intensive treatment. We agree that all fibrotic oedema are resistant to tonometers, but consider that not all resistant oedemas are fibrotic. A tough oedema could be because water is imprisoned in the very small and tight extracellular compartments that generate a very strong pressure. Other authors [13] sustained that *"A harder swelling is dominated by adipose tissue and can be treated with liposuction, while the softer one is treated conservatively"*.

7. THE MULTI-FREQUENCY BIO-IMPEDANCE

Multi-Frequency Bio-impedance Analysis (MFBIA) is a physical application of a derivation of Ohm's law. It allows us to measure the total water content of the body, both intra and extra cellularly. MFBIA can be applied to the quantification of unilateral lymphoedema. MFBIA involves passing an extremely small AC current at frequencies from 4 kHz to 1MHz, through the body and measuring the opposition to the flow of this current (defined as impedance). At low frequencies, current passes through the extracellular fluid (ECF) space and does not penetrate the cell membrane, characterized by the theoretical resistance at zero frequency (R0). At high frequencies, however, the current passes through both the intracellular fluid (ICF) and ECF.
Based on this concept, together with the fact that the impedance of a geometrical system is related to conductor length, cross sectional area and signal frequency, a value of impedance can be calculated from a current passed through the body. The measured impedance is inversely proportional to the amount of fluid. By appropriate choice of signal frequency, this can be made specific for ECF or for total fluid determination.
MFBIA analysis of a limb requires that the electrodes be placed at the extremities and the root of the limb. There are various methods for doing this, either at predetermined anatomical sites like the wrist and shoulder [14], or as some authors have proposed at a determined length (40cm apart) [15].
These machines were created for whole body measurements with electrodes placed at the hands and feet. When dealing with lymphoedema because the electrodes are placed specifically to measure a limb, the majority of results for the different volumes indicated on the screen of the machine cannot be interpreted. The impedance is the only data that can be exploited, since the other values are calculated by an algorithm, which takes into account the BMI, statistic data of fluid volume determined by isotope dilution for the whole body, and anthropometric parameters from peer-reviewed published journal articles.
The liquid content of a normal limb and more specifically an oedematous limb varies depending on different variables: temperature, relative humidity, physical activity, diet, variations in the adipose tissue, alternation of decline and orthostatic position, tight clothing, the dominant arm, elastic sleeves, metabolism, and hormonal cycles to name only a few. Because of the changes in liquid content, impedance also varies from one measure to the next. We need to considerate these aspects in the methodology of studies, despite the fact that the results obtained may be erroneous or at least disputable.
When dealing with lymphedema, the use of MFBIA can be considered when taking into account the flaws described above. Then, we concentrate on the impedance ratio between two limbs, the presumed affected and non affected limb, at a precise moment in time.
Even though MFBIA is less time consuming than water immersion methods, it provides us with a global overview that doesn't indicate towards which anatomical area the interstitial part of the oedema is being displaced.
MFBIA is more sensitive than circumferential measurements at low levels of oedema and so it can be used to discriminate between affected and non affected limbs. This method of

measurement can be useful for detecting the beginning of oedemas, enabling the rapid installation of focused treatments in order to reduce consequences of lately diagnosed, and therefore treated, oedema. Further prospective and multicentric studies are necessary to validate and expand the use of this preventative methodology.

8. QUALITY OF LIFE SCALE

Lymphoedema decreases the quality of life of affected patients and up until recently has been neglected. Consequently little data on this subject is available. Generally studies use the SF-36, a generic quality of life instrument [16], more recently specific questionnaires relating to quality of life with lymphoedema are being or have been validated such as the FACT-B questionnaire and LYMQOL[28].
R. Launoix et al. suggested an original ladder : l'ULL-27 test [17] defined by 27 items and 3 dimensions. Physical (14 questions), Psychological (7 questions) and social (6 questions). Its validity is debated in the principal articles which specify its construction and utilization's method.

9. VEINOUS ECHOCOLOR DOPPLER SCAN

Lymphoedema are always associated with a more or less important venous insufficiency, for this reason echocolor Doppler needs to be integrated in the assessment of lymphoedema. The echography is an undisputable examination for investigating lymphnodes. Some authors have demonstrated that is also possible to visualize large lymphvessels by means of echography using linear probes of 15 MHz [18].

10. THE SUB-CUTANEOUS TISSUES ECHOGRAPHY

This inexpensive and non invasive examination allows us to appreciate the fibrosis and the local thickness of the oedema and overall the thickness of the dermis involve in the dermal backflow. High-resolution cutaneous ultrasonography makes it possible to differentiate lymphoedema from lipoedema [19].
This imaging tools can be useful in the case of functional limitation without notable volumetric deterioration. Actually, volumetry does not show the underlying muscular waste when it is masked by an oedema. By means echography authors has describe that it seems that there exists a preclinical "pathological" state, where the thickness of the dermis increases and the fat tissue is modified without visible increases in the volume of the limb [20]. These results raise hope that a prevention approach of lymphoedema is possible using echography, but further prospective studies still have to be achieved.

11. THE LYMPHOSCINTIGRAPHY

This technique of imagery makes it possible to appreciate the performance of a lymphatic network and to reveal its topographical anatomy. It permits us to locate and to appreciate the function of the collateral lymphatic pathways, the dermal backflow, the lymphatic clearance of a certain spot, and the lymphnodes activity… It constitutes an invaluable help for the diagnosis [21], and the golden standard examination for the evaluation of lymphoedema. The examinations' result must be interpreted by a nuclear physcian specialized in the lymphatic field.
The method of injection (sub-cutaneous or intradermal) [22] is important and should be closely regulated as they influence the results greatly. Typically, the traditional protocols include injecting directly into the first inter digital space. A supplementary injection at the root of the limb, followed by a manual drainage on the potential drainage pathways enables us to visualize the functional pathways being used by the body to drain the lymphoedematous limb.
This information provides the therapist with clinical evidence so that they can adapt and base their treatment on facts.
According to the invasive aspect and the cost of a lymphoscintigraphy it doesn't appear judicious to propose it in first intention when a lympho-veinous oedema appears.
On the other hand, after a cycle of a dozen physical treatments, if the results are disappointing [5] it's useful to carry out a lymphoscintigraphy to clarify the problem by identifying the presence or not of lymphnodes and the routes of evacuation.

12. CT SCAN

The CT scan is not routinely proposed for the development and the evaluation of the lymphoedema because the cost / profit ratio is very unfavorable. The analysis of the cross sectional area allows us however to differentiate between the volume of the muscular mass compared to the oedema, and therefore to identify eventual muscular wasting [23].

13. MRI

MRI whitout contrast furnish an iconography in honey combs of the oedema. Examinations with contrast products by injection of Gd- DTPA only allow the visualization of large lymphatic vessels. More recently authors consider that MR Lymphangiography at 3.0 T provides very high spatial resolution and anatomical detail of normal and abnormal peripheral lymph vessels [24]. MRL may thus become a valuable tool for microsurgical treatment planning and monitoring.
This technique enables us to appreciate the relative composition of the oedemas as well as the eventual muscular wasting. TSE T1 and TSE T2 magnetic resonance images, provide a higher resolution imaging to distinguish the components of the oedema. MR spectroscopy permits us to calculate the relative proportions of water and fat, and this is interesting to evaluate the evolution of the composition of the oedema [25].

14. MEDICAL ELASTOGRAPHY

Is an emerging diagnostic tool for the assessment and real-time colour display of tissue elasticity. This technique based on the elastic answer of a tissue subjected to ultrasonic waves, provides a cartography of the elasticity of an anatomical area with a

millimetre-length resolution in 3D. This innovating non invasive method might be able to contribute to the study and evaluation of fibrosis of lymphoedema, therefore complementing studies with tonometry.
Results of preliminary study on lymphoedema arm and leg subcutis [26] demonstrate that at least *"in some cases it is feasible to generate poroelastograms from different lymphoedematous tissues in vivo. They also suggest that lymphoedematous tissues exhibit a temporal poroelastographic behavior that is significantly different from the behavior that characterizes normal tissues. Thus, poroelastographic techniques may be of use in the diagnosis and evaluation of lymphoedema"*.

CONCLUSION

Our overview is certainly not an exhaustive paper, but aims to investigate briefly the most frequently available techniques to assess lymphoedema.
The challenge that the clinician faces nowadays in treating lymphoedema is finding the adequate tools to evaluate the situation and to make the right decisions regarding the treatment, not only in function of the volume, but taking into count other parameters as well.

The most accessible techniques beside the obvious cost benefits do have their advantages. In the case of segmental lymphoedema (referring to the segment of a limb), the measuring tape method remains the easiest and comparatively most sensitive method; it is sensitive to the perimeter, graphically reproducible, and gives clear evidence of the displacement of the oedema.
Concerning the hands and feet, the immersion technique is the most adequate, while for flat surfaces, the caliper provides the easiest information concerning the thickness of the skin.

More sophisticated techniques require training, and can be less accessible on a routine basis. Never the less these techniques can reveal much more information than the naked eye. The most available of these techniques is the echography: with this we can visualize the thickness of the dermis, and of the sub cutaneous space where the oedema is situated. Because lymphatic diseases are often connected with venous insufficiency the echocolor Doppler is an indisputable tool in the diagnostic of phlebo-lymphoedema insufficiency.
MRI and CT-scan remain reserved for scientific research or particular complex clinical case. Other than in particular cases this approach cannot be used in a routine basis, because of their high cost and limited availability.

The whole body laser scanner is a promising new perometer, but quite expensive and still requires more research to evaluate its interest in the field of lymphology.

Bio impedance analysis appears interesting but procedures need to be validated to evaluate the intrinsic qualities of measurements, like the sensitivity and the resolution in order to use this system to detect lymphoedema before its visible onset.

Thermography was abandoned twenty years ago but nowadays with new advances in technology it has become an affordable and easy to use piece of equipment to evaluate the skin thermogram throughout the evolution of the oedema.
Lymphoscintigraphy remains the golden standard to visualize the physiology and physiopathology of the lymphatic system. It is now being used in synchronization with a scanner that enables a 3D visualization. By means of additional injection at the root of the limb, it is possible to visualize the pathways used and therefore direct the hands of the physiotherapist to work in accordance with evidence.

Another aspect that is often put in second place is the quality of life and also the functional impact of lymphooedema27. For example, we have only found one study that investigates gait analysis in lower limb lymphoedema, and validated questionnaires about quality of life remain rare.
Finally it is becoming more and more apparent that a myriad of techniques are available to evaluate lymphoedema, so we are hopeful for the future, knowing that possible combinations of these techniques will enable us to progress in the diagnosis, treatment and research of lymphoedema.

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23rd International Congress of Lymphology

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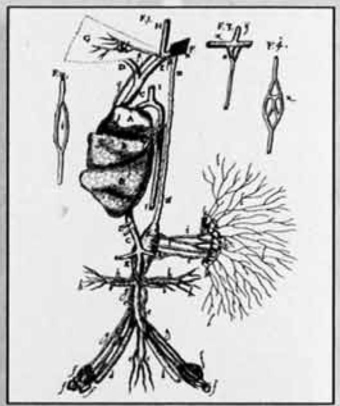
Welcome to Sweden and the 23rd International Congress of Lymphology!

Dear Colleagues and Lymphologists!

The Department of Plastic and Reconstructive Surgery at Malmö University Hospital, Sweden, is honoured to organize the 23rd International Congress of Lymphology in cooperation with the Faculty of Medicine at Lund University, and the Swedish Lymphology Association. Problems related to the lymphatic system are central issues for us, and one of our main focuses is the development of surgical techniques related to lymphology.



Olof Rudbeck (1630-1702), a Swedish scientist, published his first treatise *De Circulatione Sanguinis* in 1652, at the age of 22 years only, and he actually became the first one to describe the delineation and function of the lymphatic system in *Nova Exercitatio Anatomica*, which he published one year later. With this historical perspective in mind, we are enthusiastic about hosting the 23rd International Congress of Lymphology in Sweden. We are also proud of being entrusted with the task of arranging the prestigious congress in the city of Malmö. In fact, Malmö connects on to another pioneering scientific work in the field of lymphology performed by Thomas Bartholin (1616-1680), who was active in the nearby capital of Denmark, Copenhagen.

In 2011, from September 19 to 23, the most renowned scientists from all over the world will gather in Malmö to present and debate their front line knowledge and experiences in the various fields of lymphology. This will assure for an interdisciplinary and all-round illumination of the lymphatic system, its pathophysiology, and the state-of-the-art of different treatment regimes. Moreover, at the end of the summer but before fall, September is an excellent time of the year to visit Sweden.

We look forward to seeing you all in Malmö on this very special occasion. Please contact us for any additional information or suggestions that can make your stay even more pleasant in our dynamic and beautiful city.

Turning Torso, a 190 m tall building for residents. Photo: Pierre Mens

On behalf of the Organizing Committee,

Håkan Brorson

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