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The founding and development of the Czech Lymphology Society

BRIEF GUIDELINES OF THE CZECH LYMPHOLOGY SOCIETY

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Dear readers,

My friend and editor of the European Lymphology Journal, Dr. Michelini, has asked me to write about the curriculum of our Czech Lymphology Society, the sister organization of the European Lymphology Society. For this collaboration, I have requested the help of members of the executive committee of the Czech Lymphology Society, my coworkers and colleagues. Here, we briefly introduce an article about the founding and development of the Czech Lymphologic Society.

DEVELOPMENT

In the last century, lymphology societies were founded in many European countries. In contrast to classical medical societies, for example the societies of surgery and internal medicine, whose foundations were placed in the 19th and beginning of the 20th centuries, lymphology societies were founded after World War II. The situation is no different in the Czech Republic. Until this time, patients with lymphedema were sporadically treated at various hospital departments and by general physicians without basic theoretic and practical knowledge.

The Czech Lymphology Society is a part of the complex of medical societies, which are united under the auspices of the Czech Medical Society of Jan Evangelista Purkinje founded in honor of the Czech, world-renown scientist-physiologist Jana Evangelista Purkinje. The Czech Lymphology Society was founded in 1992, at the European Lymphology Congress GEL, which took place on September 18-19th, 1992 in Prague. At the GEL Congress, Dr. Bechyne, Prof. Bruna, Prof. Eliska, Prof. Elisková initiated the founding of the Czech Lymphologic Society. The Czech Lymphologic Society is an interdisciplinary society, which unites physicians and health care personnel – physiotherapists, lymphotherapists, and nurses working in various fields – whose work involves the care for patients affected by various forms of lymphedema. From a clinical perspective the physicians and lymphotherapists are represented in the fields of angiology, dermatology, oncology, gynecology, and surgery. From the diagnostic specialties, these representatives are health care professionals in nuclear medicine and radiology, in the theoretical fields those in anatomy and pathology. The Society was encompassed into a complex of specialized medical societies, whose head organ is the Purkinje Medical Society. The society was not founded without lymphology experience. Members of the society extended the work of Czech physicians and scientists, who

dedicated their work to lymphology after the World War II from 1960-1990 and were renowned worldwide. Mostly, these were members and highly effective research workers of the world lymphology society (Prof. Malek, Prof. Belan, Prof. Bartos, Prof. Brzek, Prof. Barinka). The first chair of the Lymphologic Society was Prof. Benda and the scientific secretary was Dr. Bechyne and in 1999 Prof Eliska. Prof. Benda held the position of chair until 2004. Afterwards, this function was taken over by Prof. Eliska and the secretary was Dr. Wald.

EDUCATION AND ARRANGEMENT

The substance of the society has developed gradually since its foundation. In the first stage, there was theoretical and practical education in courses of lymphology and lymph drainage. The courses were practiced and legalized under the Institute for the Continuing education of doctors and nurses in Prague and in Brno. The main topics are theoretical lectures in the subjects of anatomy, physiology and pathology of lymphatic vessels and nodes, which supplement clinical lectures on the diagnosis and clinical signs of lymphedema. An important part is the practical instruction of lymph drainage and bandaging. The course is terminated by examination by a committee. As long as the society has been in existence, there has been a constant increase in the flood of information on lymphology, which is then presented in subsequent 3-4 day courses and also at congresses of the Czech Lymphology Society to society members. The congresses take place every year, and last 2 days with a dual subject matter: on the one hand, in the form of typical classic lectures, and on the other, in practical demonstrations. One afternoon of the congress is dedicated to the practical portion. Even lymphology specialists from abroad are invited to lecture. After founding the society and after courses were held, lymphology centers were and are being founded in

hospitals, mostly at departments of dermatology, and also in private practice. They are operated by course participants. The head of such a center is always a physician who has completed a course in lymphology.

PROBLEMS

From this perspective, it seems that the situation regarding Czech lymphology is very good. But it is a long way from exemplary. Currently, we consider it a handicap, that in the Czech Republic there is no hospital facility in the form of a lymphology department (clinic). In the Czech Republic, there is no surgical facility that has a systematic focus on reconstructive surgery of lymphatics. Only in some cases, are debulking procedures performed and in selected cases treatment by liposuction has been initiated. Controversial questions and problems regarding insurance payment for such procedures are being discussed. Several of the health care professionals instructed at the courses do not practice lymphology. There is even a problem with the development of a genetic laboratory for the diagnosis of primary lymphedema. We are aware that there are many things still to improve. Recently, members of the executive committee have developed guidelines of the Czech Lymphology Society for the treatment of lymphedema. They are brief guidelines, which serve for orientation for the society members, and further for the ministry of health and insurance companies. Here, we briefly introduce these guidelines, so that the members of the European Lymphology Society will gain insight into our society.

Brief guidelines of the Czech Lymphology Society

Program of quality and standard therapeutic measures

LYPHHEDEMA - Standard treatment plan

Definition of the disease:

Lymphedema is a high-protein edema, which develops from the accumulation of macromolecular substances and interstitial fluid due to dysfunction of the lymphatic system and inadequate proteolysis. It is the result of disrupted lymphatic drainage of tissues while there is normal or disturbed capillary filtration.

Disease classification:

Classification of lymphedema according to clinical stage:

- 0 stage **latent lymphedema** is a condition in which lymphatic drainage is disrupted and decreased, but there is no clinical manifestation of edema.*
- 1st stage **reversible lymphedema** is intermittent edema, in which the balance between resorption and transport of interstitial fluid is disrupted, with consequent stasis of lymph and accumulation of proteins in the interstitium.*

*2nd stage **irreversible lymphedema** is edema, in which there is permanent disruption of the balance between resorption and transport of interstitial fluid with consequent stasis of lymph and accumulation of proteins in the interstitium.*

*3rd stage **elephantiasis** is monstrous lymphedema whose basis is chronic lymphatic insufficiency associated with deforming fibrotic-sclerotic restructuring of the skin, subcutaneous tissue, and other tissues of the affected region.*

Classification of lymphedema according to etiology:

- 1. Primary** (congenital-dysplasia of the lymphatic system)
 - non-familial
 - familial.
- 2. Secondary**
 - benign: iatrogenic (postoperative, post-radiative), post-inflammatory, parasitic, posttraumatic, artificial
 - combined edema of mixed etiology (i.e. phlebolymphe^dema, lipolymphe^dema, myxedema)
 - malignant: by compression or invasion of primary tumor or metastases into the lymphatic system

The basis of the structural and functional pathological changes

If the products of tissue metabolism are not cleared away by the lymphatic system even after utilizing all compensatory mechanisms, insufficiency of the lymphatic system develops resulting in a pathological condition, which is called lymphedema. A discrepancy develops between the transporting capacity of the lymphatic vessels and the amount of lymph produced in a given unit of time. There is insufficient clearing away of proteins, which accumulate in the interstitium, and are the cause of chronic inflammation of the skin, subcutaneous tissue, and other tissues in the affected region. Chronic inflammation sets off a cascade of reactions of various cell proliferations in a time interval of even several years. In the tissues, fibroblasts are activated, collagen fibers proliferate, which results in fibrosis and sclerosis of the skin and subcutaneous tissue. There is proliferation of adipocytes with consequent fatty degeneration. In the epidermis, there is proliferation of epidermal cells, which leads to hyperkeratosis and papillomatosis. The number of melanocytes also increases and as a result the amount of melanin increases. There are ectasias of lymphatic vessels, which lead to the development of fistulas and cysts. There may be hyperplasia, which in its final stage of proliferation can result in tumorous growth (fibrosarcoma, liposarcoma, basocellular skin carcinoma, lymphangiosarcoma, and possibly even melanoma). Since lymphedema most often affects the lower and upper extremities, stasis of lymph and interstitial fluid with all its negative consequences affects all structures of the extremity, meaning the skin, connective tissue, blood vessel walls, nerves, ligaments, tendons, muscles, and joints. The result is fibrosis and sclerosis of the skin and subcutaneous connective tissue, worsened metabolism of the blood vessel wall, edema and worsened function of the motor apparatus, which adds to the decreased mobility of the extremity due to its increased weight and volume.

Epidemiological characteristics

In the Czech Republic, every 12th women is affected by breast cancer annually. In approximately 40% of these women, lymphedema develops in connection with the comprehensive treatment and the actual malignancy itself. The incidence of lymphedema in association with tumors of the head and neck and tumors of the pelvis is currently around 10%.

The epidemiologic incidence of primary lymphedema is not precisely known. It is estimated at 10-15% of cases of lymphedema of the extremities. The basis of the disease is genetic aberration of the chromosomes with a dysfunction of lymphatic growth factors. Primary lymphedema might not be diagnosed during an entire lifetime, as long as it does not manifest clinically (latent or reversible stage of lymphedema).

Qualifications required

Institutions

Lymphologic facilities, especially outpatient clinics, are included in the framework of health care facilities. Stemming from the interdisciplinary character of the field, the character of provided care is determined by the needs and range of outpatient and inpatient facilities, which function in specific regions.

Specialists

The structure of specialists caring for patients with lymphedema consists of:

- A/ **physicians-lymphologists** with a specialization in one of the main medical fields, and who underwent certified specialized courses with an examination and adequate experience, in the future physicians with additional specialization in lymphology.
- B/ **lymphotherapists**- health care professionals with secondary and higher education (rehabilitation, nursing), who underwent certified specialized courses with an examination, and work under the supervision of a physician- lymphologist.

Technical requirements

In agreement with the standpoint of the Ministry of Health of the Czech Republic and Health Insurance, the technical equipment of lymphologic facilities is divided into two groups:

- A/ **diagnostic equipment** for measuring volume of the extremity, radionuclide lymphoscintigraphy, ultrasound examination, and including equipment for multipurpose examination at radiology facilities (i.e. MR, CT, PET), genetic and other facilities.
- B/ **therapeutic equipment** multi-chamber equipment for sequential lymphatic drainage and others.

Other requirements

Spatial requirements- examination room, room for therapeutic procedures. Cooperation with other specialized health care facilities (angiology, surgery, oncology).

Process of care

Entry criteria in the process of care

Patient history – clinical status:

To diagnose disorders in lymphatic flow, lymphostasis and the development of lymphedema, in clinical practice in the majority of cases, it suffices to **evaluate the patient and family history, basic medical examination (including laboratory results) and physical examination of the extremities by inspection and palpation.**

These examinations have the goal of demonstrating the presence of edema in the extremity and eliminating other causes of edema other than stasis of lymph (venous insufficiency, cardiac, nephrotic, hypo-proteinemic edema, lipedema, cyclic edema and others).

Lymphedema is characterized by cool, pale, painless edema of the extremity, at first soft and pasty, gradually tougher to hard, which appears either in the distal parts of the extremity (primary lymphedema), from where it spreads proximally, or below an obstruction in the lymphatic circulation, usually in the proximal regions, from where it spreads distally. The edema has a progressive character. Specific diagnostic significance in lymphedema of the lower extremity is given to so-called Stemmer's sign (marked thickening of the skin and subcutaneous tissue on the dorsal surface of the 2nd toe and usually also big toe of the extremity).

The clinical signs of the disease are dependent on the type of lymphedema, its duration, coincidence with other diseases and number of recurrences of secondary infection (erysipelas).

Patient entry criteria

Positive history
Typical clinical signs
Subjective complaints
Results of basic and supplementary examinations

The actual process of care

Entry diagnostic examination

Specific history
Physical examination
Supplementary imaging examinations
Laboratory screening
Genetic examination as required

Measuring the volume (circumference) of the extremity is part of the physical examination. Even other modern methods, i.e. photometric, computer, and others can be considered for measurements. If after carefully performed initial examination, the diagnosis of lymphedema is not sufficiently accurate, additional information can be obtained from other, specifically imaging examinations.

Among the most beneficial is **triphase radionuclide lymphography** (lymphangioscintigraphy, LAS), which allows the evaluation of the morphology of the lymphatic network in the extremities, as well as the transporting function of the lymphatic circulation.

Differential diagnosis:

If lymphedema affects only one extremity, it is not caused by diseases of any organs (heart, kidneys, myxedema, hypoproteinemia, etc.). Chronic lymphedema is constant, which differentiates it from angioneurotic and cyclic edemas. If the

edema is associated with lengthening of the extremity, especially at a young age, it is necessary to consider Klippel-Trenaunay syndrome. Lipedema (more precisely lipohyperplasia+lipohypertrophy) is edema that always affects both extremities, thus bilateral, where the consistency of the subcutaneous tissues is typical for accumulation of fatty tissue. It may be problematic to differentiate lymphedema and edema of the extremities in venous insufficiency.

These two pathologic factors may be mutually combined.

Radionuclide lymphography (LAS) has a deciding influence in the differential diagnosis, clearly eliminating or demonstrating the role of disordered lymphatic circulation on the developed edema.

Other supplementary examinations include imaging by magnetic resonance, computer tomography or ultrasound (with utilization of the duplex Doppler signal). These examinations are highly specialized and should be performed in specialized facilities with sufficient experience.

Treatment

Therapy should always be provided in a facility of specialized care – lymphocenter, in which the requirement of collaboration of a lymphotherapist and physician-lymphologist and also the basic technical requirements of the facility are fulfilled. Collaboration with a psychologist is beneficial. Only in such cases, can patients be provided with comprehensive therapy. This consists of:

A/ Complex decongestive physical therapy

- manual lymph drainage
- intermittent compression treatment- machine lymph drainage
- compressive therapy
 - one- and multi-layered bandages
 - compressive stockings
- physical and breathing exercises
- hygiene of the skin and life style
- complementary physical therapy (hydro-balneotherapy and others)

B/ Supportive care

- pharmacotherapy (proteolytic enzymes, flavonoids and others)
- psychotherapy

C/ Surgical treatment (in indicated cases)

- causative
 - derivative
 - reconstructive
- symptomatic
 - liposuction
 - resection procedures
 - debulking

D/ Treatment of the complications of lymphedema

- A/ Dermatological – prevention and treatment of erysipelas – adequate care of small erosions, treatment of interdigital mycosis, treatment of focal infections of streptococcal and staphylococcal origin.

B/ Orthopedic

C/ Neurologic

D/ Oncologic

E/ Mental a social

Time plan of treatment

Treatment should be initiated as early as possible. It takes place in two phases/stages.

In the initial stage of intensive **reduction** of lymphedema, daily treatment is required for 6 weeks.

In the **maintenance** phase, regular care at home is required (the patients may receive equipment for lymph drainage, they may be taught the basic technique of manual auto-lymph drainage, or possibly a family member may be instructed, daily administration of compressive stockings- bandages and exercise is necessary), in some cases outpatient care for maintenance therapy at a lymphocenter with follow-up at longer time intervals.

Lymphedema mainly requires lifetime care. In the case of worsening of clinical condition, it is necessary to repeat the reductive phase and then to continue with the subsequent maintenance therapy.

The program of a rational treatment strategy is determined by a physician specialist- lymphologist.

Surgical treatment is performed at specialized facilities.

Conditions for terminating the process of care

Finishing patient criteria

In rare cases of lymphedema, in various time frames, there may be disappearance of clinical signs of edema, in some cases even complete normalization of function of the lymphatic system. In these cases, it is possible to terminate treatment. However, permanent follow-up is required.

Prognosis

Lymphedema is a chronic disease, which can be significantly influenced by therapy. **Generally, the earlier the diagnosis, the more timely the initiation of treatment in the appropriate quality and duration, the better the prognosis.** However, that also depends on the degree of fibrotic changes of the subcutaneous tissues and on patient compliance. Nevertheless, after discontinuation of therapy, in most patients there is a gradual recurrence of the initial condition. Primary lymphedema is a lifetime disease and only regular and adequate treatment can prevent the development of serious complications and disability. In secondary lymphedema, in various time intervals there may be complete disappearance of edema. The effect of treatment depends on the cause of edema (worse prognosis in postoperative edema with lymphadenectomy for breast cancer or pelvic tumors, and post-radiation edema, very good prognosis in the majority of posttraumatic edemas or after by-pass surgery).

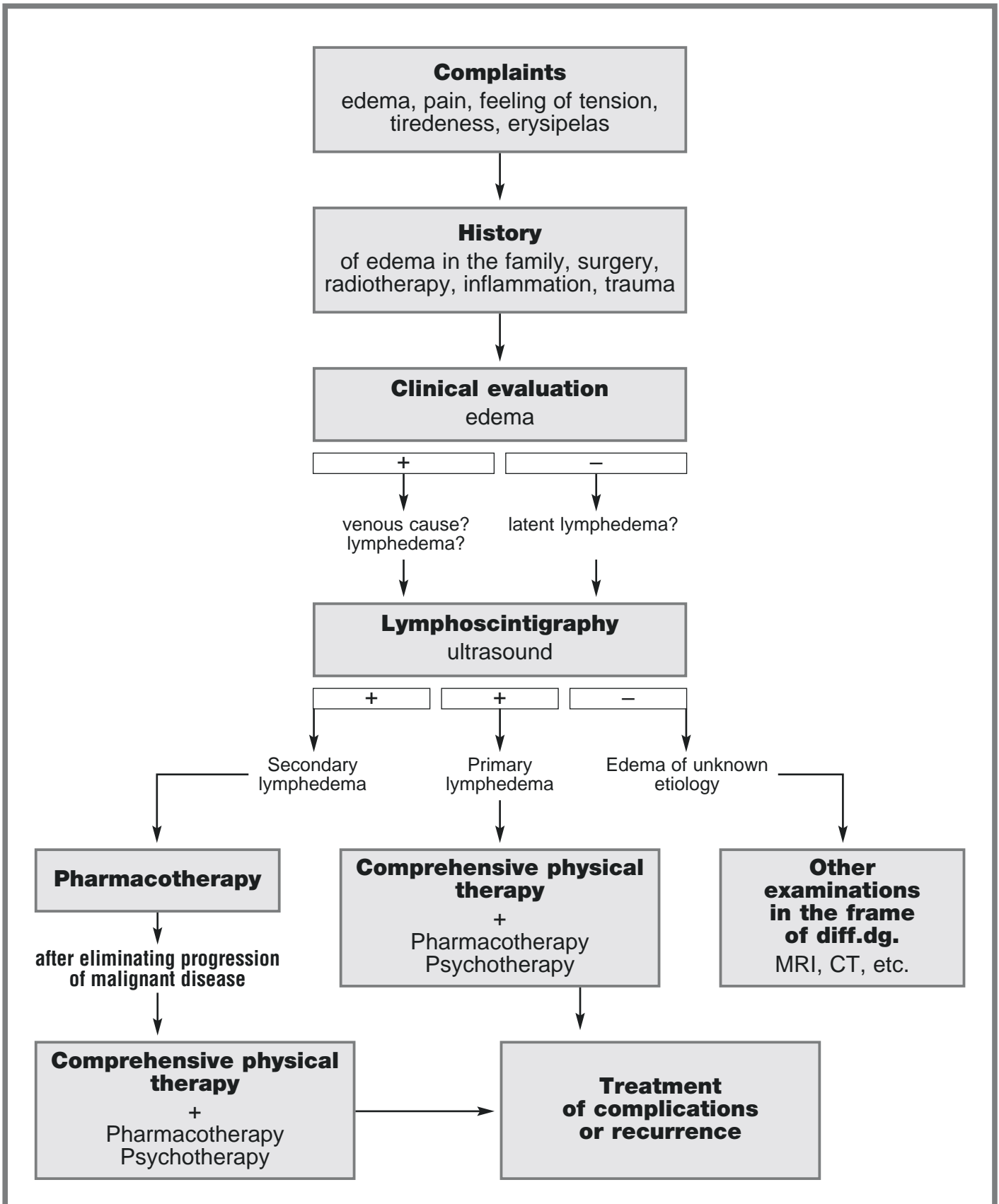
Results – criteria and indicators of quality of care see diagrams

Diagrams

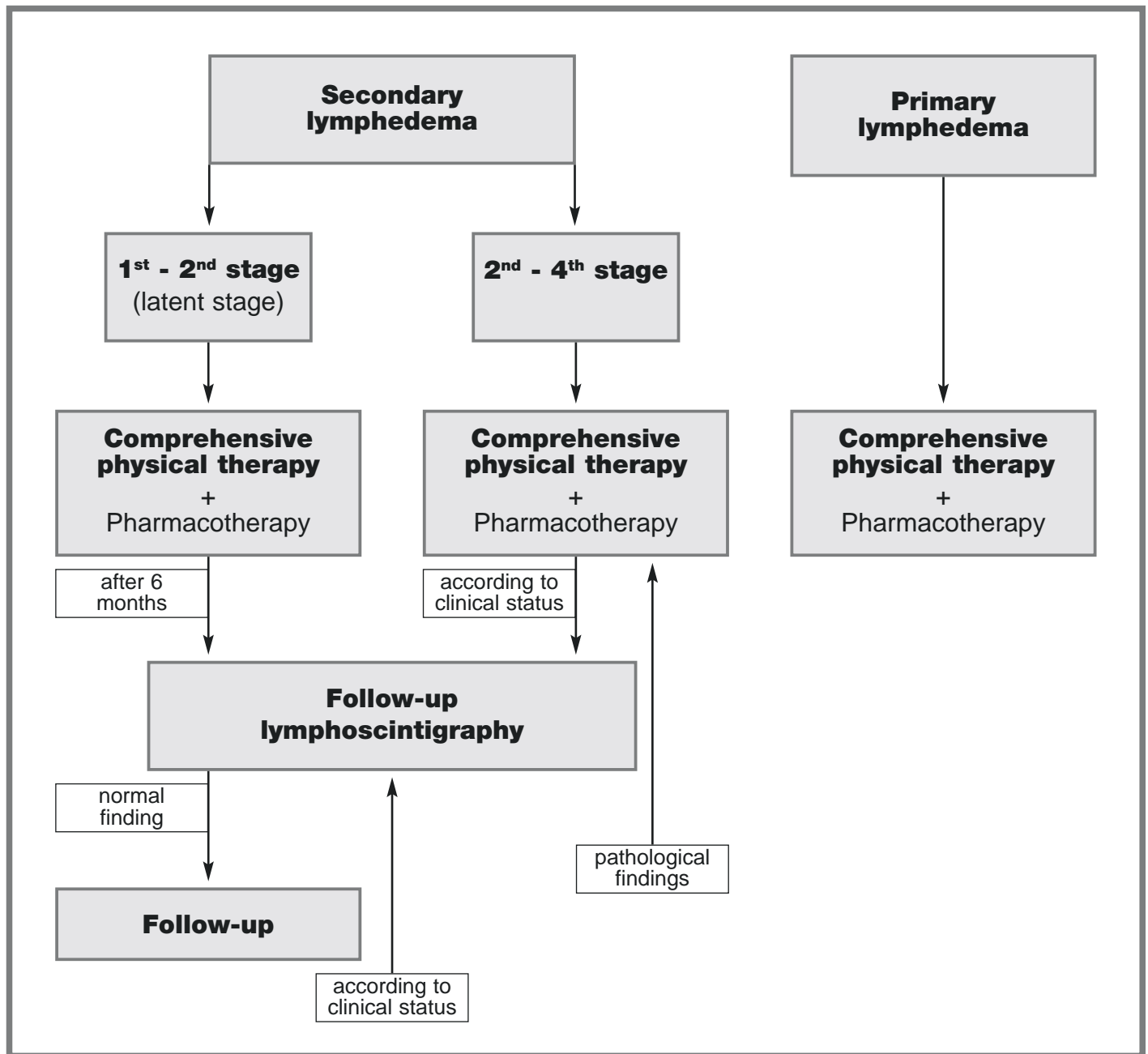
1/ Algorithm of determining the diagnosis of peripheral lymphedema

2/ Algorithm of therapy and monitoring of peripheral lymphedema

Algorithm for determining the diagnosis of peripheral lymphedema



Algorithm of therapy and monitoring of peripheral lymphedema



FUTURE

To colleagues in various European states, the guidelines mentioned above may seem clear, and thus not requiring mention. There may also be other opinions regarding this topic. From the perspective of our Society, which is a young society, these guidelines were developed to be understood by physicians as well as lymphotherapists. They reflect our current work. With time, they will certainly be supplemented and amended. The classification of lymphedema given here corresponds to the basic classic classification, which are comprehensible and routinely used by all lymphologists worldwide. We are aware that our work also

has some inadequacies, they were mentioned above with the intention and aim of improving our work. Viewing the work of our society retrospectively, we can state that step by step, even if with difficulty, there is a slow and gradual progress is taking place. There is even an increase in lymphologists who attend congresses of the European Lymphologic Society. All authors mentioned above believe in improving the work of the society and are trying to achieve this goal.

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LYMPH NODE DERIVED LYMPHATIC ENDOTHELIAL CELLS: ISOLATION, PURIFICATION, CHARACTERIZATION AND LONG TERM CULTURE

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ABSTRACT

Aims. To contribute to the study of lymphatic endothelial cells (LECs) biology, our aim is to purify and characterize LECs isolated from human lymph node.

Method. Using a two-step purification method based on the sorting of endothelial cells with CD31 coated beads followed by purification with monoclonal antibody D2-40, we were able to purify and *in vitro* expand, for up to 8-10 passages, human derived LECs from lymph node. Cells were analyzed for phenotypic and functional properties.

Results. LECs specifically express lymphatic markers and, when seeded on Cultrex BME, were able to form a capillary-like network.

Conclusion. We propose here a new technique to make available ready to use source of abundant well-characterize human lymph node derived LECs to examine normal profiles and behaviour to compare with abnormal conditions.

KEYWORDS: lymphatic endothelial cells, lymphatic markers, cell purification, D2-40, lymph node.

INTRODUCTION

The lymphatic system complements functions of the blood vascular system by regulating tissue fluid balance, facilitating interstitial protein transport and serving immunological function. This system collects the extravasated protein-rich fluid and lymphocytes from the tissue and reintroduce them into circulation becoming essential for the continuous removal of interstitial fluid and proteins (Ryan and Curri, 1989). By directing leukocytes and antigens from tissue to lymph node, lymphatic system play an essential role in initiating immune response (Pepper and Skobe, 2003). Furthermore the importance of the lymphatic system as a pathway for metastasis has been well recognized. Metastasis of most cancers occurs initially through the lymphatics and the extent of lymph node involvement is one of the most important prognostic indicators of patient outcome (Hartveit, 1990; Pepper

and Skobe, 2003). Given their central role in all the function above mentioned it is surprising that lymphatic endothelial cells (LECs) have until recently been poorly characterized. Lymphatic vessels were initially identified by the absence of erythrocytes in their lumen and by the presence of less elaborated cell junctions, as compared with blood vessels (Gerli et al., 1990; Erhard et al., 1996; Leak, 1970). LECs also have lower levels of CD34 and von Willebrand factor (vWf) expression than blood endothelial cells (BECs). Finally, LECs growth is selectively regulate by VEGF-C, a member of the Vascular Endothelial Growth Factor (VEGF) family (Ferrara and Davis-Smyth, 1997; Veikkola et al., 2003), via VEGF receptor-3 (VEGFR-3) (Clauss, 2000; Ferrara, 2002). Very few attempts have been made in the past to isolate LECs but this has recently become possible thanks to the identification of such new lymphatic markers as transmembrane mucoprotein Podoplanin (Matsui et al., 1999), hyaluronidase receptor Lyve-1, (Banerji et al., 1999), VEGFR-3 (Jussila and Alitalo, 2002), transcription factor Prox-1 (Petrova et al., 2002) and the D2-40 monoclonal antibody (mAb), which recognizes O-linked glycoprotein (Khan et al., 2002). As already demonstrated for BECs (Page et al., 1992; Thorin and Shreeve, 1998; Turner et al., 1987), LECs share certain common functions, but it is also now clear that considerable structural and functional heterogeneity exists depending on the length of the lymphatic vascular tree and in the microvascular lymphatic beds of various organs from which are derived from (Garrafa et al., 2005). In the present study we describes a new method for the purification, characterization and *in vitro* expansion of human derived LECs from lymph node (Ln). The availability of Ln-LECs will permits to analyze their molecular and functional characteristics and will provides novel insight into the molecular basis of lymph node function.

MATERIALS AND METHODS

Processing of lymph node fragments

Human lymph node fragments were obtained from patients undergoing therapeutic surgical procedures, according to the principles listed in the Helsinki Declaration. Lymph node

fragments, finely minced with scissors, undergo enzymatic digestion for 3 h at 37°C with 0,25% (w/v) collagenase/dispase solution (Boehringer Mannheim, Mannheim, Germany). The resulting digestion product was filtered through a 30 mm pore size filter and the cells washed and cultured in T25 flask coated with collagen type I and fibronectin, in the presence of Endothelial Growth Medium (EGM, BioWhittaker, Walkersville, MD).

LECs isolation and culture

Once at confluence, cells from primary cultures were resuspended and incubated with magnetic beads (Dynal, Oslo, Norway) coated with antibody anti-CD31 (Sigma-Aldrich, St. Louis, MO; cell/bead ratio 1:2). Total endothelial cells (ECs) positive to mAb-CD31 were recovered by using a magnetic particle concentrator and cultured in the presence EGM plus VEGF-C (50 ng/mL) (R&D System Inc., Minneapolis, MN). Subsequently LECs were positively purified from total ECs, once at confluence, using magnetic beads (ratio cell/bead 1:5) coated with D2-40 monoclonal antibody (mAb, Signet Laboratories, Dedham, MD) and then seeded onto collagen type I and fibronectin coated wells and cultured in the presence of EGM added with VEGF-C (50 ng/mL).

Immunocytochemistry

Immunocytochemical studies were performed on LECs seeded on glass slides coated with collagen and fibronectin, and then fixed in cold 4% paraformaldehyde in PBS, pH 7.4, for 10 min at room temperature. Cells were washed twice with PBS, incubated with 10% goat serum (Gibco, Grand Island, NY) to block aspecific binding, then incubated for 90 min at 37°C with mAb to CD31 (dilution 1:100; Dako) and to CD44H (dilution 1:40; R&D System Inc., Minneapolis, MN), D2-40 (dilution 1:160), or with rabbit antisera to vWf (dilution 1:80; Sigma-Aldrich), Lyve-1, Podoplanin and Prox-1 (dilution 1:160; Reliatech, Braunschweig, Germany). For UEA-1 staining, cells were incubated with biotin-conjugated UEA-1 and then with a rabbit anti-UEA-1 antibody (Sigma). The complex was revealed using cyanine dye-labeled anti-rabbit IgG. Air-dried cells were then mounted with Fluorosave (Calbiochem, La Jolla, CA) and photographed using a Zeiss Axiophot-2-microscope (Oberkochen, Germany).

Cord formation on Culture Basement Membrane Extract (BME)

LECs were seeded at a concentration of 105/well in EGM plus VEGF-C in 8 wells chamber slide previously coated with 100 µL of Cultrex BME (10 mg/mL, Biodesign International, Saco, MA). Cord formation was obtained after few hours of incubation and routinely examined by light microscopy.

RESULTS

Isolation and culture of LECs

Specimen obtained from lymph node fragments were processed and subjected to enzymatic digestion. The resulting cultures produced a heterogeneous adherent cell population consisting mainly of elements with an endothelial morphology. To allow

purification of ECs from processed tissue, we used magnetic beads coated with antibody anti-CD31, followed by a second step consisting in the selection of LECs from CD31 positive population using coated beads with mAb against the specific lymphatic marker D2-40. LECs obtained as described above were cultivated in presence of EGM containing VEGF-C (50 ng/mL) on collagen and fibronectin coated flasks. Under microscopic examination, LECs showed an elongated shape, typical of ECs with a prominent nucleus (Fig. 1). Cells were serially subcultured at a split ratio of 1:3. The number of serial culture propagations did not increased and never exceeded 8-10 passages.

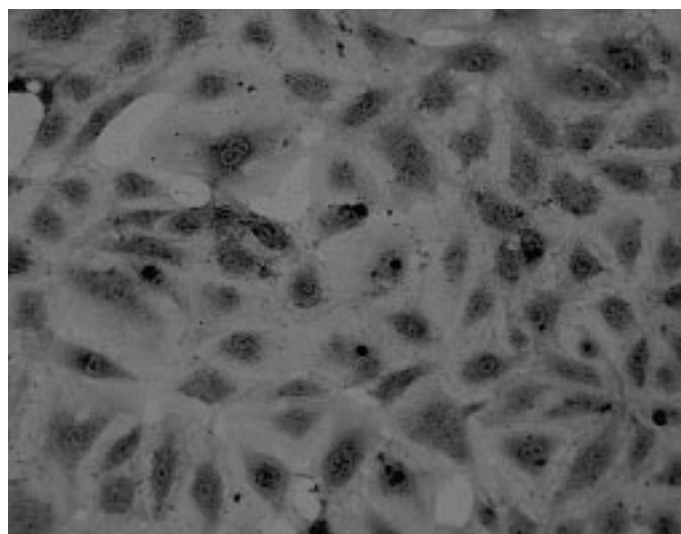


Fig. 1: Ln-LEC CD31+/D2-40+ monolayer.

Immunocytochemistry

As shown in Table 1, Ln-LECs were strongly stained by Prox-1 mAb and Lyve-1 mAb. D2-40 and Podoplanin were also well expressed. Lymphatic specific markers were absent on HUVEC (data not show). Ln-LECs were strongly positive for UEA-1, express at lower intensity CD31 and CD44H and were almost negative for vWf. Vascular specific markers were all well expressed on HUVEC (data not shown).

D2-40	Lyve-1	Podopl.	Prox-1	CD31	vWf	Ulex 1	CD44H
++	+++	++	+++	++	+/-	+++	++

Tab. 1. Expression of lymphatic and blood vascular markers on Ln-LECs.

Cord formation on Culture Basement Membrane Extract (BME)

When subjected to tube-forming assay in 8 wells chamber slide previously coated with 100 µL of Cultrex BME, Ln-LECs were able to form tube-like structures in presence of EGM plus VEGF-C, after 12-24 h of incubation. (Fig. 2)

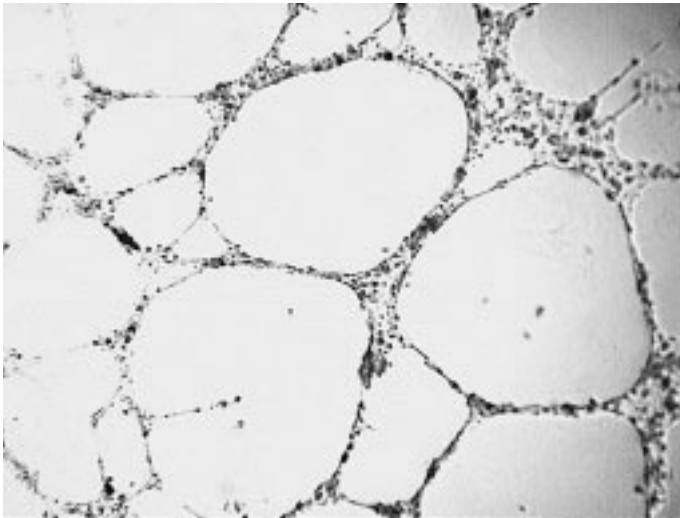


Fig. 2: Tube forming assay. Ln-LECs were able to form tube-like structures on Cultrex BME.

DISCUSSION AND CONCLUSION

Many attempts have been made in the past to isolate and culture LECs from a variety of specie. LECs purification and growth have become a reality only recently, since the discovery of specific lymphatic growth factors and substrates (Makinen, 2001; Kriehuber et al., 2001; Garrafa et al., 2006). In this work, we describe a useful method that allows the isolation, propagation, and characterization of LECs prepared from lymphatic-rich tissue such as lymph node. Generation of LECs required tissue dispersion, removal of non-adherent cells after 12-18 hours of plating, an initial immune preselection with CD31-coated magnetic beads, followed by second selection with magnetic beads coated with D2-40 mAb. Ln-LECs were morphologically similar to BECs, although their shape appeared more elongated. Most of the known blood vascular markers, such as CD31 and vWf, were present at low levels on their surface in agreement with previous studies of derma-derives LECs (Kriehuber et al., 2001). Lymphatic markers were well represented on Ln-LECs. Infact these cells are strongly stained by Prox-1 and Lyve-1 and, consistent with the report by Schacht et al., who recently demonstrated that Podoplanin is recognized by the D2-40 have similar expression of this two markers. Moreover, Ln-LECs culture are able to form a capillary-like network when seeded on Cultrex BME, indicating their capability to form lymphatic vessel *in vitro*. Therefore we were able to culture Ln-LECs in the presence of specific growth factors and extracellular matrixes without losing any of their different properties and functional activity. In conclusion the main indication yielded by our results, there, is that lymph node fragments are a valuable source for isolating human lymphatic endothelial cells that may provide new tools for investigating and understand the role in the physiological and pathological events that involve lymph node such us immune response and lymphatic tumor metastatic dissemination.

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RESPONSES TO TREATMENT IN PRIMARY AND SECONDARY LYMPHEDEMA: EFFECTS OF BODY MASS INDEX AND DELAYS IN RECOGNITION

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ABSTRACT

Untreated or unrecognized lymphedema is associated with progressive disease (1), and recognition of this disease can be more difficult in obese individuals. We retrospectively evaluated the records of 23 patients with primary lymphedema and 58 patients with postsurgical secondary lymphedema to evaluate the relationships between delays to therapy after onset of symptoms (DTRX) and body mass index (BMI) with severity of symptoms and responses to treatment. In patients with primary lymphedema, DTRX was not found to be significantly associated with clinical variables measured before or after treatment and also was not associated with objective or subjective assessments of response to therapy. However, patients with secondary lymphedema demonstrated correlative relationships between DTRX and severity of lymphedema. Increased BMI was associated with lesser asymmetries in limb circumferences both before and after treatment, and also with longer DTRX in secondary lymphedema. All patients (100%) with either primary and or secondary lymphedema demonstrated both subjective and objective responses to therapy, but slightly greater responses were observed in secondary lymphedema. Lymphedema continues to occur after lymph node dissection (LND) (2,3). Delays in seeking treatment after onset of symptoms are associated with more severe lymphedema, and patients with increased BMI tend to seek treatment later in the course of their disease.

KEYWORDS: Primary Lymphedema, Secondary Lymphedema, Body-Mass Index, Therapy, Manual Lymph Drainage Therapy.

INTRODUCTION

The recognition and treatment of lymphedema of the extremities is often interrelated with assessments of obesity and regional adipose distributions. Both patients and caregivers experience difficulties in separating the effects of each process, which are often concomitant. This may lead to delays in diagnosis, which can

allow lymphedema to progress, and increase the likelihood of secondary complications including infection (4).

Although refinements of surgical technique and sentinel node procedures have reduced the morbidity of LND (5), lymphedema of both upper and lower extremities still occurs after LND. This investigation was therefore undertaken to analyze the relationships of delays in seeking treatment and body mass index (BMI) to severity of disease and responses to treatment in patients with primary lymphedema and secondary postsurgical lymphedema (2).

METHODS

Records of 81 adults with lymphedema were retrospectively reviewed. The patient population consisted of 23 patients with primary lymphedema of the legs (11 with bilateral and 12 with unilateral involvement) and 58 patients with postsurgical secondary lymphedema of the arms (n = 32) or legs (n = 26). Patients with acute or active infection, trauma or recurrent malignancy were not included in this study. Patients with primary lymphedema had a mean age of 39.4 years, range 16 – 75, and those with secondary lymphedema had a mean age of 56.2 years, range 24 – 87.

Delays in seeking treatment or delays to therapy (DTRX) were measured in weeks from the time of onset of symptoms until the time of first treatment in all patients, including patients with postsurgical secondary lymphedema. BMI was calculated as weight in kilograms divided by the square of height in meters.

Subjective symptoms of pain, heaviness and pressure of the involved extremity were rated on the scale of 1-10 (from least to most severe, respectively). The circumference of biceps and palm in the upper extremity patients and upper thigh and ankle were recorded in centimeters. Symptoms and circumferences were recorded before the initial treatment session and immediately after the 15th treatment session. The 15 sessions of therapy usually were completed over a period of approximately one month.

The treatment protocol consisted of manual lymphatic drainage (MLD), followed by gradient sequential intermittent compression pump at 30 mmHg with a soft sleeve incorporated into the pump and stretching exercise program. The patients were fitted with a compression elastic sleeves or stockings for daily use after the first session. All patients also were instructed in, and utilized, the following home therapies: skin care, elevation of the affected limb, self massage and exercises tailored for the affected limb. The patients wore the elastic compression sleeves during the day.

Severity of disease and both subjective and objective responses to therapy were analyzed with regard to DTRX and BMI. Spearman rank order correlation coefficients were determined to evaluate potential relationships between severity of symptoms versus DTRX and BMI. Reductions in size were treated as positive numbers, and hence rank order correlations would be positive when another variable, such as DTRX or BMI increased as limb size diminished. Pearson correlation coefficients were not employed, since symptoms could be ordinally ranked but not parametrically determined, and since DTRX were approximated based on patients' histories. BMI, age, reductions of limb circumference, and DTRX were compared in groups with primary and secondary lymphedema using two-tailed unpaired t-tests.

RESULTS

Comparative features of the groups with primary and secondary lymphedema are presented in Table I. Comparing the groups with primary and secondary lymphedema, the patients with primary lymphedema were a younger population than the group with secondary postsurgical lymphedema, $p < 0.001$. The American Obesity Association and the U.S. department of Health and Human Services classify a BMI of over 25 as overweight. Approximately half of all patients demonstrated BMI over 25. No significant differences were observed in DTRX and BMI among the groups with primary and secondary lymphedema (both $p > 0.2$). Patients with primary lymphedema were younger ($p < 0.001$), and demonstrated greater reductions of limb circumferences than patients with secondary postsurgical lymphedema ($p < 0.001$).

Primary Lymphedema: Clinical variables investigated included the severity of lymphedema, as manifested by asymmetries in limb circumferences both before and after therapy, and responses to therapy as manifested by percentage reduction in circumference of the affected limb as well as subjective changes in symptoms with

Table 1. Type of Lymphedema.

Patient Characteristics	Primary	Secondary
Number	23	58
Age, Mean (years)	39.4	56.2
Age Range	13 – 75	24 – 87
BMI (Kg/m ²), Mean	24.9	24.7
BMI over 25	48%	50%
DTRX (weeks), Mean ± SD	117 ± 161	67 ± 138
Reduction, Mean ± SD [†]	7.8 ± 2.6%	5.1 ± 2.4%

treatment. These clinical variables were correlated with DTRX and BMI using Spearman rank order correlations. Results of these determinations are presented in Table II.

In the 23 patients with primary lymphedema, DTRX was not found to be significantly associated with any clinical variables measured before or after treatment and also was not associated with objective or subjective assessments of response to therapy (all $p > 0.1$). BMI demonstrated a significant inverse correlation with asymmetry in ankle circumferences both before and after therapy (both $p < 0.02$), indicating that more obese patients demonstrated less relative asymmetry in ankle circumferences. However, BMI was not found to be related to measurements of response to therapy in primary lymphedema. Patients with increased BMI responded to treatment no better or worse than those of more asthenic habitus. Nevertheless, all patients with primary lymphedema (100%) demonstrated improvements of both quantitative measures and subjective symptoms, although their responses were independent of DTRX and BMI (all $p > 0.1$). DTRX and BMI were not themselves correlated in patients with primary lymphedema.

Secondary Lymphedema: Clinical variables investigated included: 1) the severity of lymphedema, as manifested by asymmetries in limb circumference both before and after therapy 2) responses to therapy as manifested by percentage reduction in limb circumference of the affected limb 3) subjective changes in symptoms with treatment. These clinical variables were correlated with DTRX and BMI using Spearman rank order correlations. Results of these determinations are presented in Table III.

Table 2. Results in 23 Patients with Primary Lymphedema.

Arm+Leg Spearman Rank Order	DTRX	BMI	
R values	% Shrinkage Thigh	0.025	-0.154
	% Shrinkage Ankle	0.155	-0.263
	Subjective Response to Rx	-0.0108	-0.237
	% Asymmetry PreRx Thigh	0.249	-0.004
	% Asymmetry PreRx Ankle	0.076	-0.558
	% Asymmetry PostRx Thigh	0.285	-0.028
	% Asymmetry PostRx Ankle	0.294	-0.485
	BMI vs. DTRX		0.0373
R values	% Shrinkage Thigh	0.908	0.478
	% Shrinkage Ankle	0.478	0.222
	Subjective Response to Rx	0.959	0.272
	% Asymmetry PreRx Thigh	0.248	0.984
	% Asymmetry PreRx Ankle	0.726	*0.006
	% Asymmetry PostRx Thigh	0.184	0.898
	% Asymmetry PostRx Ankle	0.17	*0.019
	BMI vs. DTRX		0.862

* $p < 0.05$

Table 3. Results for 58 patients with Secondary Lymphedema.

Arm+Leg Spearman Rank Order		DTRX	BMI
R values	% shrinkage arm or thigh	0.271	0.099
	Subjective Response to Rx	0.0539	0.139
	%Asymmetry PreRx	0.324	0.337
	% Asymmetry PostRx	0.255	0.372
	BMI vs. DTRX		0.322
R values	% shrinkage arm or thigh	0.04	0.46
	Subjective Response to Rx	0.687	0.296
	% Asymmetry PreRx	0.0134	*0.00988
	% Asymmetry PostRx	0.0537	*0.00415
	BMI vs. DTRX		*0.0141

* p < 0.05

Longer DTRX were associated with greater limb asymmetries before therapy ($r = 0.324$, $p < 0.02$) and a tendency toward this was also seen after therapy ($r = 0.255$, $p = 0.0537$). In addition, it was also associated with greater objective responses to treatment, manifested by greater volume reduction of the affected limb ($r = 0.271$, $p < 0.04$). Increased BMI was associated with greater asymmetries in limb circumferences both before and after treatment (both $p < 0.001$), but not with limb volume reduction after treatment.

All patients (100%) demonstrated both subjective and objective responses to therapy. Subjective assessments of responses were not found to correlate with DTRX or BMI however. Objective measures of response to therapy demonstrated significant variation with DTRX, but not with BMI. As mentioned previously, patients with secondary lymphedema demonstrated lesser reductions in limb circumference with treatment than did those with primary lymphedema ($7.8 \pm 2.6\%$ in primary versus $5.1 \pm 2.4\%$ in secondary, $p < 0.001$). Patients with larger BMI however, demonstrated longer DTRX ($r = 0.322$, $p < 0.02$). They also demonstrated greater asymmetries of limb circumference both before ($r = 0.337$, $p < 0.01$) and after therapy ($r = 0.372$, $p < 0.01$).

CONCLUSIONS

In patients with primary lymphedema, we did not identify relationships between DTRX or BMI with more severe disease or with responsiveness to therapy. More obese patients demonstrated less asymmetry in ankle circumferences before and after therapy (both $p < 0.02$), but this could relate to the absolute larger dimensions of their ankles, making asymmetries more difficult to recognize. The relatively smaller number of patients ($n = 23$) studied with primary lymphedema may also limit the statistical power of any analyses of that data. Since asymmetry was relatively less pronounced in more obese patients, recognition of lymphedema could be more difficult in these individuals, both for patients and for caregivers.

Based on clinical findings and lymphoscintigraphy (Fig. 1), 12 of the 23 patients with primary lymphedema were diagnosed with unilateral disease. Results were not different in these subgroups, although the small numbers could affect statistical power for detecting small differences.

Patients with secondary lymphedema demonstrated a correlation of DTRX with greater asymmetries of affected limbs before treatment and stronger relationship between DTRX and reductions of limb circumferences after treatment. This suggests that the disease progressed with time in the absence of treatment. There was also a tendency for DTRX to be related to greater limb asymmetry after treatment that was close to being statistically significant ($p = 0.0537$). Progression of disease can increase the likelihood of additional problems such as infection and progressive cutaneous fibrosis (6). This again emphasizes the need for patient education after LND, in order that lymphedema can be promptly recognized and treated (7). In general, patients with secondary lymphedema were slightly less responsive to treatment than those with primary lymphedema, as measured by changes in limb circumference ($p < 0.001$).

BMI was also found to be correlated with longer delays in seeking treatment. Similarly, BMI correlated with greater limb asymmetry both before and after treatment, whether due to delayed recognition, lesser symptoms, or for other reasons. Increased BMI did not correlate with increased volume reduction after treatment. These observations underscore the need for patient education about early signs and symptoms of lymphedema after surgery that affects lymph node basins, so that lymphedema can be timely diagnosed and treated (8).

Responsiveness to treatment was not found to be related to BMI. Nevertheless, all patients with both primary and secondary lymphedema responded to treatment.

Lymphoscintigraphy was useful in clarifying diagnoses, providing improved delineation of the relative contributions of obesity, identifying congenital deficiencies of lymphatic structures, depicting the extent of unilateral or bilateral involvement, and identifying the presence and severity of secondary lymphedema (9,10).

DISCUSSION

Patients with primary lymphedema demonstrate variable clinical presentations. Patients with increased BMI demonstrate less apparent asymmetry in the size of the lower extremities both before and after treatment. Therefore, recognition of lymphedema requires meticulous attention. Patients' responses to therapy and symptoms were relatively independent of BMI; however, all patients improved with treatment. Lymphedema continues to occur after LND (3,11). Delays in seeking treatment after onset of symptoms are associated with more severe secondary lymphedema. Patients with increased BMI tend to seek treatment later in the course of their disease, and demonstrate greater asymmetries in limb circumference. Healthcare providers should educate patients who undergo LND to recognize the early symptoms of lymphedema, particularly heavier patients, in order that they seek treatment earlier in the course of the disease.

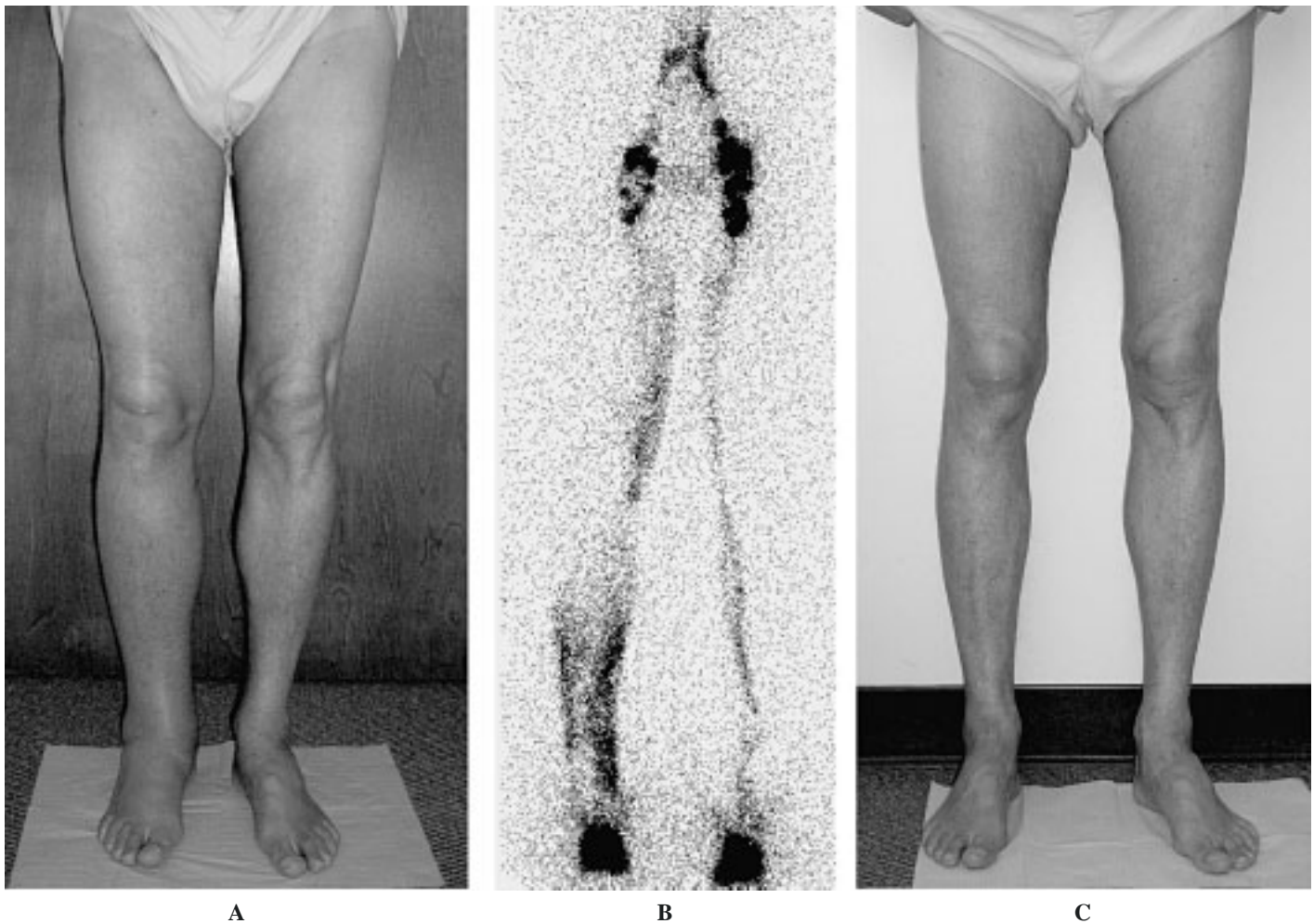


Figure 1. Forty-four year old male with right lower extremity secondary lymphedema after right inguinal lymphadenectomy.
(A) Lymphedema, Stage I.
(B) Lymphoscintigraphy shows a partial absence of the right inguinal lymph nodes and dermal back flow in the right lower extremity, distally more evident than proximally.
(C) Results after 15 sessions of MLD treatments.

One methodological issue should be addressed regarding this study. Patients with unilateral and bilateral primary lymphedema were grouped together, which may have decreased the ability to detect significant relative changes of asymmetry, although it would not affect the results for comparative reductions of single extremities.

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EFFECT OF RUNNING AND WALKING ON THE PRESSURES AT THE INTERFACE BETWEEN ELASTIC STOCKINGS AND THE SKIN

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ABSTRACT

The aim of the present study was to evaluate in a dynamic study the affect on running and walking on the working pressures of elastic stockings.

Pressure variations at the interface between elastic stockings and legs were studied dynamically. An elastic stocking manufactured by the company Selecta with gradients of 20/30 and 30/40 were utilized according to the size of the legs of each subject who participated in the study. An apparatus calibrated to measure pressures at half-second intervals was used.

Walking and running cause different pressure variations at the interface between elastic stockings and the leg. Walking produced smaller variations compared to running, which gave pressure peaks of greater than 100 mmHg.

In conclusion, there are variations in pressure between elastic stockings and the leg both when walking and running transforming almost stable pressures in the standing position, to pressure variations with high peaks. Running produces greater alterations than walking and gradients of stockings also affect the variations of the pressures.

KEYWORDS: Dynamic study, walking, running, elastic stockings, working pressure.

INTRODUCTION

The utilization of elastic stockings is a modern method of applying pressure which has as one of its advantages its simplicity of use. Efficiency depends on the pressure exerted and its adaptation to the limb. Elastic stockings function by exerting a constant external pressure on the limb favoring both lymphatic and venous drainage.^(1,2)

The European Committee for Normalization classifies stockings into four groups, Class I, II, III and IV, depending of the pressure exerted at the ankle. Class I exerts a pressure of from 15 to 21 mmHg; class II pressures of 23 to 32 mmHg, class III pressures of 34 to 46 mmHg and Class IV greater than 49 mmHg. It is important to choose the class of stockings by considering the underlying pathology and the targeted system.^(1,3,4)

It is well known that compression therapy is efficient in the rehabilitation of patients with lymphovenous diseases when it is indicated and adapted to the needs of each patient. In these cases elastic stockings are a good therapeutic option, although it is important to be aware of the pressure gradients of specific materials in order to guarantee the efficiency of treatment.⁽¹⁾ Nevertheless, there are no publications that have reported dynamic evaluations of these stockings.

The aim of this study is therefore to assess the affects of walking and running on the working pressure variations of elastic stockings in a dynamic study.

METHOD

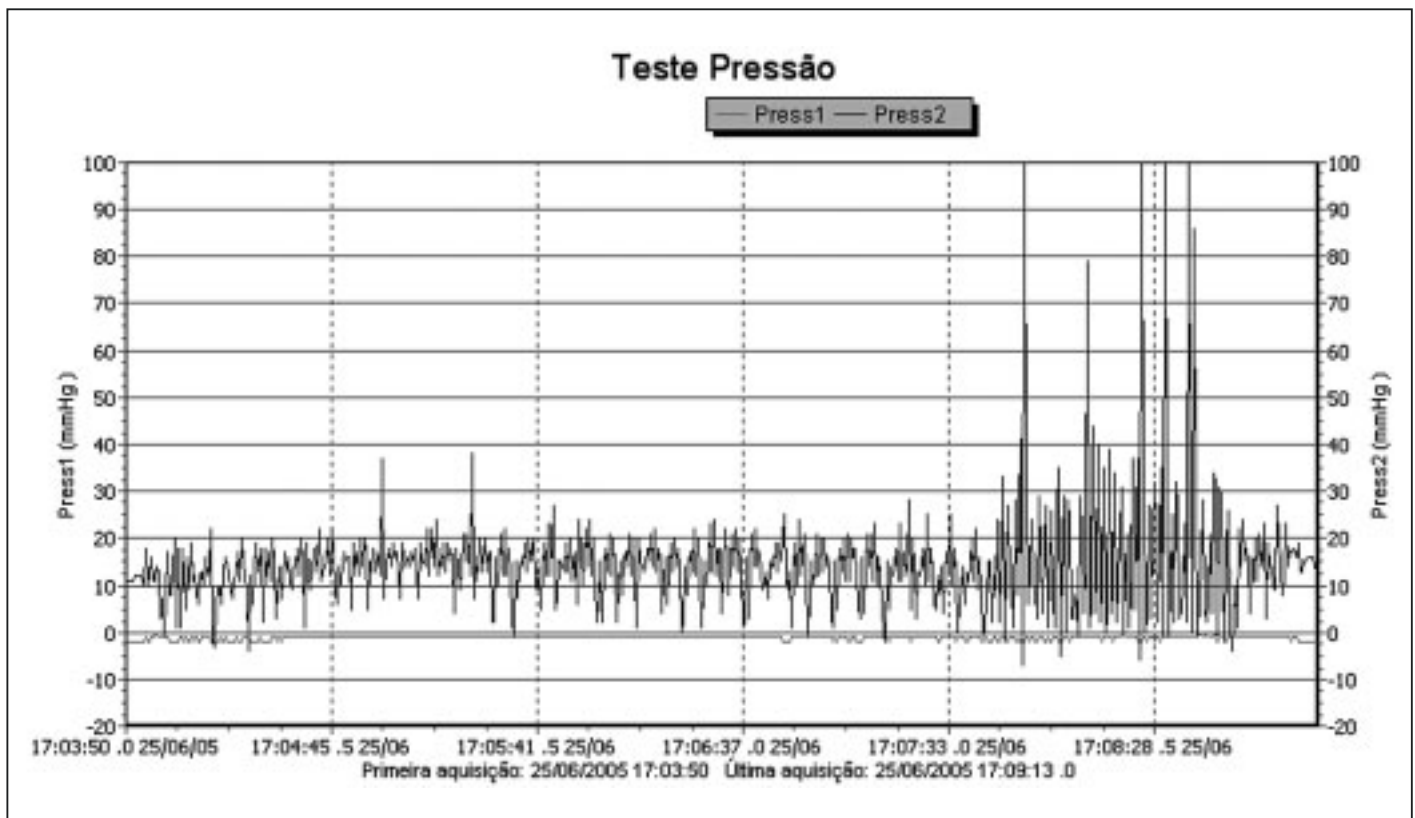
Pressure variations between elastic stockings and the skin were measured in dynamic studies in a total of 30 events involving one male and two female subjects. Elastic stockings manufactured by the company Selecta with graduations of 20/30 and 30/40 were utilized depending on the size of the participants' lower limbs. None of the participants reported venolymphatic diseases. To measure the pressure variations, an apparatus developed by Godoy and Braille in Braille Biomedica, São José do Rio Preto, Brazil was employed. The apparatus was programmed to measure the pressure at half-second intervals and it could be used in both dynamic and static studies. A pilot test was made to determine the best site for the sensors and the medial region of the calf muscle was chosen. Before each event, the apparatus was calibrated with the individual in the standing position and subsequently the subject ran three times around a block and walked one time around 400 meters. The pressure variations of each event were recorded. The experiment was repeated ten times for each of the three participants, five times per leg.

RESULTS

In every events walking and running caused different pressures variations at the interface between elastic stockings and the skin. Illustrated in Graph 1 pressure variations over time between an elastic stocking and the skin during walking and running with graduations of 20/30 and graph graduations of 30/40. There is a drop in the pressure from the initial static standing position when the individual started to walk or run. Walking produces smaller variations when compared to running.

DISCUSSION

This dynamic study demonstrated the affect of walking and running on the pressure variations at the interface between elastic stockings and the skin. No publications on dynamic studies under these conditions were found in the MEDLINE Electronic Library, even though differences in pressure are of fundamental importance in respect to the mechanisms pertaining to the use of elastic stockings. There are only small variations in the standing position whereas with walking and running these variations increase. The compression pressures (gradients of the stockings) in the standing position produce different pressures during muscle movement. Another finding is that the mean pressure drops during walking and even further when running and the pressure returns to the



Graph 1. Illustrates pressure variations over time between an elastic stocking and the skin during walking and running with graduations of 20/30. The first section is walking the second walking and finally running again.

initial pressure of the standing position when these exercises are ceased.

The findings suggest that activities that utilize a greater impact of the limbs, such as when running, may benefit more using stockings with lower gradients and even so very high working pressures are reached. Graph 1 demonstrates that from initial pressures of 12 mmHg, pressures of greater than 100 mmHg are reached. These data are important to guide sportsmen who use elastic stockings when training. The utilization of elastic stockings during exercise may be useful, specifically with patients suffering from venolymphatic diseases however, each case should be individually analysed.

CONCLUSION

In conclusion, walking and running affect the pressure differences at the interface between elastic stockings and the skin, transforming almost stable pressures in the standing position to

great variations of peaks and valleys. Running causes greater differences than just walking and the gradients of the stockings also affect the size of the variations.

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THE SKIN TEMPERATURE UNDER MULTILAYERED BANDAGES

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ABSTRACT

Purpose: multilayered bandages (MLB) coupled with manual lymphatic drainage is the main axis of the physical treatment of lymphedema. The physical principles which underlie the therapeutic efficiency of the MLB have not been clearly defined especially with regard to the thermal effect.

It seems evident that the skin temperature increases when it is covered by MLB. Currently no study has described the value and the variation of that temperature. This study aims at defining values for the cutaneous temperature under MLB for patients who suffer of a secondary lymphedema of the upper limb.

Method: the skin temperature of both upper limbs was measured and compared during 24 hours for 12 patients wearing a MLB. It was measured every minute with temperature sensors fixed on the skin and connected to a data logger.

Results: the skin temperature increased rapidly and reached 34,49°C - SD ± 070°C during the whole period where the limb was covered by the MLB.

KEYWORDS: multilayered bandages, skin temperature, temperature sensor, physical treatment of lymphedema.

INTRODUCTION

The wearing of multilayered bandages constitutes one of the main axis of the intensive physical treatment of lymphedemas. (1,2,3)

The treatment consists of covering the affected limb with superimposed bandages of very different types.

The careful association of these materials provides this specific bandage with a mechanic reaction which distinguishes it from the mechanic reactions observed for each material on its own.

Its resistance opposes itself to the inherent variations in volume linked to the contractions of the muscles and arterial pulsation.

This generates very important variations in pressure at the interface of the skin-bandage creating thus a continuous massage at high pressure and great amplitude.

The mechanic principles which underlie the therapeutic efficiency of the multilayer bandages have been the subject of some studies. (4,5) The thermal principles, however, have been largely ignored. The use of heat as a therapeutic vector to reduce the oedemas has been essentially developed in China and in Japan where secondary lymphoedemas caused by filariasis infection are very common. (6) The affected limb is heated by wrapping humid and hot tissues around it.

This low cost and relatively efficient therapeutic approach used for the reduction of the volume of secondary lymphoedemas caused by filariasis has been transposed, using new technologies, to other secondary lymphedemas as well as to primary lymphedemas.

The heating is produced with various physical means such as the exposure to infrared lights, to electromagnetic waves (7) or vibrations of high frequency which heat the tissue via cavitation. The advocates of these techniques (8) affirm that "the temperature of the heated skin with its subcutaneous tissue of the diseased limb is increased, its metabolic activities are reinforced, and the lymphatic regeneration and lymphatic return are restored to meet the therapeutic goal (9)

The evident risks of necrosis and burning of the skin have resulted in the non inclusion of this therapeutic approach in the international consensus document of the physical treatment of lymphedema. (1)

Studies on the measurement of the heating of limbs submitted to such techniques have shown that regardless of the physical means used, even if in certain systems the temperature surrounding the limb reaches 80 °C, the increase of the cutaneous temperature never exceeds 4°C. (9)

The efficiency of multilayer bandages for the reduction of the volume of lymphoedemas does not need to be demonstrated anymore. Nevertheless, the physical and physiological mechanisms which preside this beneficial effect remain little explored considering their widespread use and dissemination. The patients who undergo multilayered bandaging describe systematically a sensation of increase of temperature in the affected limb.

This perception of heat causes sometimes sufficient discomfort to interrupt and terminate the treatment.

The evolution of the cutaneous temperature in relation with a multilayered bandage has not been studied yet.

No data exists, neither for the values of temperature reached, nor on their circadian evolution.

If the effect of the heating linked to the use of multilayer bandages is deemed useful, and even if it were harmful (10), it might be appropriate to identify the parameters in order to optimise the physiological effects.

This study aims at defining values for the cutaneous temperature under MLB for patients who suffer of a secondary lymphoedema of the superior limb.

2. MATERIAL AND METHOD

2.1. Population

The study targets a group of women who are undergoing physical treatment for a lymphoedema of the upper limb, secondary to the axillary dissection.

The sample was constituted by 12 persons selected at random from a list of patients undergoing treatment. Average age = 38 to 78 years ($54,87 \pm 12,77$ years).

We have excluded those patients with an infection, fever, a dermatological pathology on the upper limb, arteriopathy, diabetes type 2, thyroid or suprarenal disturbances.

All patients were requested to sign a document expressing their consent to participate in this study.

Material

The multilayered bandages were composed of three kind of materials.

Just over the sensor from bottom to the surface: Tricofix® One layer – Soffban® three layer – Comprilan® twelve layers all product are from BSN medical

Temperature sensor: The skin temperature is measured with surface sensors fixed on the skin of the patients: temperature sensors OM-40-C-LT. (Fig. 1)

Technical characteristics: range 0 °C to 44 °C – accuracy: $\pm 0,36$ °C at 20 °C – resolution $\pm 0,2$ °C at 20 °C – response in stirred water: 1 min.

Data logger: The two temperature sensors were connected with a small mobile data logger including a third temperature sensor and a humidity sensor to record the room temperature.

Technical characteristics of the data logger: 2 channels (2 sensors) - dimensions 30X50X10 mm - Firm MIE datalogger.

Data Logger software MIE datalogger for OM-40-C-LT version 3.7.3 permits to program the beginning and the end of the recording as well as the frequency of measures to be taken (we have chosen one measurement per minute).

All data are exportable in an Microsoft® Excel file.

Statistics: Statistics were performed with Statistica® Statsoft version 5.1



Fig. 1

Method

- The temperature sensors are put in place ten minutes before the MLB at room temperature. The data logger is connected to the Laptop and programmed for a recording session of 24 hours with a measurement recorded every minute, which results in 1440 measures for each limb. The measurement and the recording start immediately after the programming and the disconnection of the data logger from the laptop.
- The temperature sensors are each fixed on the side of each forearm. Les sondes de températures sont fixées sur chacun des avant-bras à leur face latérale. (Fig. 2)
- The MLB is put down in a standardised manner for all patients using the materials described above and respecting the number of stratum for each material.
- The data logger is fixed on the surface of the MLB in order to measure the room temperature as well as the humidity of the room on the outside of the MLB. (Fig. 3)



Fig. 2



Fig. 3

- The patient then leaves the treatment center. The patient is requested to pursue her activities as usual and to return to the center after 24 hours in order to take off the bandage and to download the data.
- The typical average and deviations of the 1440 measures obtained by each sensor are calculated and registered in the table below. These values which are analysed with a T-test for dependant samples and 11 DOF determine the degree of significance of the difference between the average temperature observed on the covered and non covered limbs.
- A graphic of the skin temperature of the two upper limbs in function of time is being drawn and analysed.

RESULTS

Main graphic obtained with the data of every patient. (Fig. 4)

The difference of the skin temperature between the bandaged & non bandaged arm

The table below shows the average cutaneous temperature and their standard deviation recorded over a period of 24 hours for each of the two arms of all twelve patients.

Fig. 5: average & standard deviation of the skin temperature measured during 24 hours for the two upper limbs of twelve patients.

Statistical analysis (Fig. 6)

- The difference between the temperature recorded on the “free” non covered upper limb and the covered upper limb is highly significant.
- The mean difference reaches nearly 2,5 °C.

- The maximal peak of the difference of skin temperature between limbs recorded was $6,85\text{ °C} \pm 0,36\text{ °C}$.
- The skin temperature of the bandaged limb never reached the central body temperature.

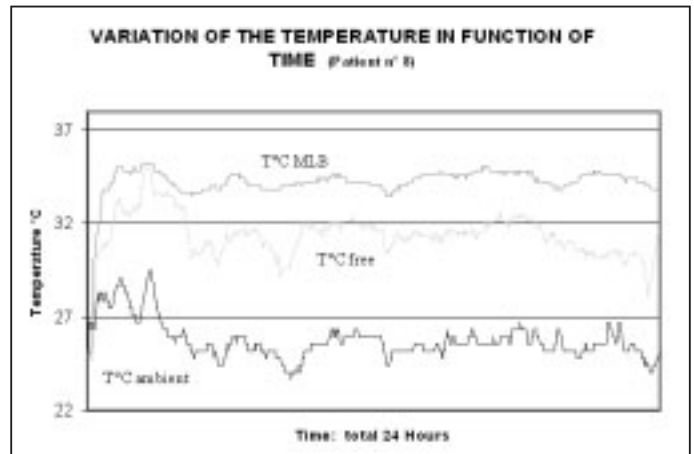


Fig. 4

N°	T°C free	SD +/-	T°C mlb	SD +/-
1	33.32	± 1.10	35.18	± 0.46
2	33.21	± 1.72	34.52	± 0.92
3	31.61	± 1.52	33.53	± 0.50
4	32.11	± 0.84	35.44	± 0.45
5	32.05	± 3.01	35.64	± 1.20
6	31.75	± 1.9	34.89	± 0.71
7	32.89	± 1.51	34.17	± 1.30
8	32.40	± 1.06	34.71	± 0.8
9	31.58	± 0.75	33.61	± 0.48
10	30.76	± 1.68	33.85	± 1.61
11	29.74	± 1.46	34.01	± 0.97
12	31.46	± 1.05	34.34	± 0.40

T°C free – Skin temperature in °C without multilayered bandages.
T°C mlb – Skin temperature in °C under multilayered bandages.

Fig. 5

T-test for Dependent Samples						
	Mean	Std.Dv.				
T°C free	31.91	1.02				
T°C MLB	34.49	0.70				
Marked differences are significant at p < .05000						
N	Diff.	Std.Dv. Diff.	t	dof	p	
12	-2.59	0.94	-9.56	11	0.000001	

Fig. 6

DISCUSSION

In our study the average skin temperature of a non covered limb remained at $32 \text{ }^{\circ}\text{C} \pm 1,02 \text{ }^{\circ}\text{C}$ in room temperature.

The mechanisms of homeothermia maintain the skin in the range of temperature as it is described in literature.

The graphics show that the skin temperature varies during the nyctemeral cycle dependent on physical activities and sleeping phases. Under the MLB those variations also exist but their range are clearly absorbed.

MLB imposes an inertia to the heat exchange system.

It acts as an thermal insulator.

The Pearson correlation coefficient calculated for each subject show that the evolution of the skin temperature is different for each arm for a same patient. These data confirm that the MLB has a physiological effect on the regulation of the skin temperature.

The mechanisms of homeothermia cannot compensate the increase if the cutaneous temperature linked to the presence of the MLB.

Although the average of the differences in temperature is about $2,59$ staggering $0,94 \text{ }^{\circ}\text{C}$ around the average, we noted numerous peaks of differences between the two limbs situated between $4 \text{ }^{\circ}\text{C}$ à $6 \text{ }^{\circ}\text{C}$, mainly during certain sleeping phases. (11, 12)

These differences in temperature could explain the difficulties experienced by some patients to tolerate the MLB during the night and the feeling of exhaustion they expressed. Indeed, the thermal discomfort could be the source of micro disruptions of the sleep which affects the quality of the sleep of these patients.

The increase of the cutaneous temperature under the MLB takes place minutes after the MLB has been put in place. During the first four hours after fitting the MLB, the cutaneous temperature is at its highest; thereafter the curb tends to decrease.

This tendency of the temperature to decrease is due to the strip of air which may ventilate the interface skin-bandage when the volume of the limb has been reduced due to the MLB. The less adherent bandage allows the air to circulate which favours the thermal exchanges and limits the insulation properties of the bandage.

This study demonstrates that the skin temperature increases rapidly and reaches $34,49 \text{ }^{\circ}\text{C} - \text{SD} \pm 070 \text{ }^{\circ}\text{C}$. During all the period where the limb is covered by the MLB, the skin temperature remains significantly higher than the skin temperature of the contra-lateral limb which is free of MLB.

The skin temperature under MLB reached the same values as described by the authors who used thermotherapy to treat lymphedema.

The MLB has the advantage of being a “thermotherapy system” wearable permanently and relatively inexpensive.

Nevertheless, the local increase of the cutaneous temperature and its maintenance over several hours may explain the discomfort expressed by some patients who have to wear an MLB.

The significant increase of the skin temperature as a result of the wearing of an MLB is not indicated for pyretic patients or for patients who have an infection or inflammation (erysipela or lymphangitis).

CONCLUSIONS

This study contributes to the studies of MLB with the aim of optimising the effects on the reduction of lymphedemas.

It proposes a thermal approach of the effects of the MLB by confirming that the skin temperature under the MLB does not exceed the central body temperature but increases nevertheless in average of about $2,5^{\circ}\text{C}$ compared to the skin temperature of the non covered limb and can attain peaks of differences of about 6°C with the other limb. The tenants of the thermotherapy dispose of a good thermotherapy system which is wearable permanently and relatively inexpensive.

The ample variations of pressure to which the lymphedemas are submitted during the use of the MLB constitute clearly one of the key elements explaining the efficiency of these specific bandages. However, it remains to be validated that the increase of the cutaneous temperature is advantageous for the reduction of lymphedemas.

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OSSEOUS LESIONS IN CHRONIC PHLEBOLYMPHEDEMA: PATHOLOGICAL PATTERN IDENTIFICATION IN MODERN MAN AND IN ANCIENT HUMAN REMAINS

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ABSTRACT

Paleopathology is by now a well established discipline with regard to applied methodology and systematic classification of acquired scientific information. Only by integrating modern medicine with the medicine of human beings of the past can both disciplines draw fruitful and bilateral knowledge contributions.

This work aims to demonstrate that paleopathological research of ancient human remains can provide very interesting information to clinical studies and investigations as concerns the pathology of chronic lymphedema.

Many aspects involving Lymphology and Phlebology are often tackled in daily clinical practice. The close correlation between lymphatic system and venous circulation begins as early as during embryo development. When, during inflammation processes, lymphothrombosis or prolonged lymphangiospasm prevent lymphatics from properly draining into the interstitial space, perivenous lymphangitis is likely to develop affecting *vasa lymphatica vasorum*. When these conditions persist, tissues begin to become fibrotic, with subsequent increase in wall thickness and stiffness of deep, communicating, and superficial vein branches. All these conditions together can, in turn, stimulate a subperiosteal bone reaction in those areas where the vessel runs close to the periosteum, with no muscle or fibrous fascial tissue in between. Bone imprints are thus formed, as if a mould were taken of vascular structures.

Through a retrospective study of current patients with phlebolympheoedema, axial tomography scans could be assessed again, thus proving the presence of similar lesions. Bone imprints of vascular origin have thus always proved to be associated with chronic vessel inflammation and to be a consequence of this condition.

KEY WORDS: Paleopathology, Phlebolympheoedema, Periostitis, Vascular lesions.

INTRODUCTION

Paleopathology is the science which investigates diseases that can be demonstrated in ancient human remains. For this reason, it has to be linked to physical anthropology on one hand and, even more, to archaeology and pathologic anatomy on the other. The methods for the recognition and investigation of ancient human remains are derived from the former discipline; collected information is then put into context with regard to historic period, geographic area, and population. Conversely, the systematic classification of lesions and diseases, diagnosis skills, and methods are taken from pathologic anatomy.

Paleopathology is therefore a borderline discipline, in between very different subjects. Providing archaeology and, as a matter of fact, history with data for an overall reconstruction of man and the features of individuals or groups of people who lived in a certain place, and in a certain, even far back, period of time makes Paleopathology so valuable (1).

Conversely, the role of Paleopathology with regard to modern medicine is less straightforward and more difficult to define. Indeed, for each disease that can be demonstrated in ancient and very ancient human remains, an equivalent modern disease can be identified. For this reason, since its inception in the first half of the 19th century, Paleopathology has looked for disease evidence in all populations on earth, at every latitude, wherever human remains could be found. Investigations are now focused on the incidence of diseases and on the difficult relation between the body and the environment, on adaptation issues, as well as on physiological and pathologic changes of man during life. Job and occupation, interpersonal relations and relations with neighboring populations, the economy and its likely impact on health conditions of individuals and groups: these are the issues covered by Paleopathology today (2).

Only by integrating modern medicine with the medicine of human beings of the past can both disciplines draw fruitful and bilateral knowledge contributions.

This work aims to demonstrate that paleopathological research of ancient human remains can provide very interesting information to clinical studies and investigations about social relations in the pathology of chronic lymphedema.

Chronic lymphedema is indeed a common disease affecting large population groups. Besides a high number of patients with clear clinical signs, there is a much more numerous group of people with sub-clinical or latent lesions, that become evident only at particular times of their life, or as a consequence of certain jobs, or life styles.

In the etiopathology of chronic, long standing lymphedema, in addition to angiodysplastic or post-trauma lesions, there is a large number of chronic, so called idiopathic lymphedemas, as well as a high amount of post-inflammation lymphedemas. In these patients, inflammation involving lymphatic vessels and lymph nodes may result out of transient or chronic, sometimes modest infection, not strictly related to traumas or wounds. Indeed, nutrition, body constitution, and severe obesity are other important factors when investigating the causes or joint causes of lymphedema onset. The very close correlation between chronic lymphedema and jobs, life styles, and behavioral and food habits of patients, as well as past infections that are either directly or indirectly correlated with the environment and features of the place, make the study of lymphedema incidence in ancient populations and in different historic and environmental contexts quite interesting.

However, collecting evidence of lesions typically found in soft and connective tissues of individuals whose skeleton only is available would seem to be quite impossible. Because of this very difficulty, paleopathological investigations have been developed aiming at providing evidence of disease through indirect signs found on the skeleton. Indeed, all physiological and pathologic changes are recorded in bones, during their slow yet lifelong remodelling. Many diseases can thus be demonstrated.

However, in order to provide reliable and scientifically correct information, a constant comparison of these lesions is required with current pathological pictures, in which the disease has been clinically and instrumentally confirmed with accurate and proven diagnostic tools.

Periostitis is a relatively common finding in Paleopathology. It can be demonstrated in a great number of individuals, if carefully searched for. It mainly affects the bones of the lower limbs and, in particular, the tibia and the fibula.

As a matter of fact, periostitis is generally diagnosed by searching for changes and the outcome of chronic inflammation on the periosteum and, in particular, for reactive sub-periosteal bone apposition. Indeed, a slight mesh of very thin bone lamellae develops on the bone surface. They are often joined or woven together to form a thin mesh, or may even grow into bigger spicules.

For many years, periostitis was thought to be the result of direct bone trauma, particularly to the tibia or malleolar regions, which are the most exposed bone portions separated from the external environment only by the skin, with no muscle tissue in between. Lesion topography - namely whether the lesions were malleolar or tibial, tibial or fibular, and, even more, whether they were lateral

or multifocal - has until now provided the diagnostic basis for the development of any epicritic reasoning.

However, such a limited etiology is unlikely to account for the high incidence of diagnosable lesions as mentioned above, especially if it is referred to sedentary populations, with irrelevant gender differences, yet with significant differences between the various age groups.

For this reason, several authors, including ourselves, have focused our attention on other etiologies, and tried to identify some useful elements for differential diagnosis (3,4,5,6).

Our research group is mainly focused on all aspects of lymphostatic disease, for this reason we have begun investigations in this direction too.

Many aspects involving Lymphology and Phlebology are often tackled in daily clinical practice. The close correlation between lymphatic system and venous circulation begins as early as during embryo development.

Lymph sacks deriving from venous formations make up the original lymph circulation structures. Also from an anatomic point of view, both circulations run above and under the fascia and are equipped with valves.

From a functional point of view, the lymphatic system plays a key role in homeostasis, in the drainage of interstitial fluid, cellular debris, bacteria, inorganic matter, higher molecular weight plasma proteins, and immune cells.

The lymphatic system is involved in every form of edema, resulting from insufficient extracellular space drainage, which may take place through two main mechanisms:

- 1) Insufficient interstitial lymph drainage (*low output failure*) due to congenital or acquired obstacle to lymph flow.
- 2) Interstitial fluid overload, with normal or increased lymph drainage (*high output failure*), such as in the case of post-thrombophlebotic syndrome and chronic venous insufficiency (CVI).

Further, lymphatic involvement in CVI becomes more severe, because of the development of dystrophic-ulcerative lesions and lipodermatosclerosis. Lymph stasis associated with CVI leads to edema which, depending on the prevailing disease, is called either phlebolympheoedema or lympho-phleboedema.

O.Eliska (7) showed lymphatic damage on the bottom and around a leg venous ulcer by taking targeted biopsies. This feature confirms the almost constant presence of perilesional edema.

It is therefore easy to understand that more insight into these close interactions between the venous and the lymphatic system is likely to promote a higher diagnostic and therapeutic success rate in daily clinical practice.

The progressive evolution of a CVI picture to trophic alterations and ulcer onset is normally coupled by a significant reduction in lymphatic drainage.

This aspect has been successfully proved by lymphoscintigraphy, with which the extent of lymphatic involvement can be assessed, and a better understanding gained of any underlying physiopathologic mechanisms. In particular, for example, lymphoscintigraphy allows investigators to accurately assess any deep lymph circulation damage during the compensation phase in post-thrombophlebotic syndrome.

Also microlymphography (8,9) has shown top dermal lymphatics deficiency, leading to lymphatic microangiopathy, characterized by obliteration and reflux.

Laser-Doppler shows reduced perilesional microcirculation perfusion, made worse by the ulcer satellite edema, all factors underlying poor ulcer scarring.

The association between lymph stasis and CVI increases the risk of dermatolymphangioadenitis, which causes edema worsening, as well as the onset of trophic skin alterations up to ulceration proper. Exudation of trophic-ulcerative lesions leads to progressive skin maceration, which is associated with other alterations such as hyperkeratosis, papillomatosis, lymphatic fistulas, verrucosis, fibrosis, all of them correlated with lymph stasis.

Further, when, during inflammation processes, lymphothrombosis or prolonged lymphangiospasm prevent lymphatics from properly draining into the interstitial space, perivenous lymphangitis is likely to develop affecting *vasa lymphatica vasorum*. When these conditions persist, tissues begin to become fibrotic, with subsequent increase in wall thickness and stiffness of deep, communicating, and superficial vein branches (Fig. 1). All these conditions together can, in turn, stimulate a subperiosteal bone reaction in those areas where the vessel runs close to the periosteum, with no muscle or fibrous fascial tissue in between. Bone imprints are thus formed, as if a mould were taken of vascular structures.

Further, lymphostasis stimulates subperiosteum bone fibrillogenesis and regeneration; these processes are normally associated with periostitis, coupled with a progressive thickening of the long bone diaphysis cortex (these conditions most often affect the lower limbs and are most evident in the leg bones). Inflammation sometimes extends to capsular-tendineous and ligament structures of the joints (most often to the tibial-tarsal joint), leading to lymphostatic arthritis, with poorly treatable bacterial involvement.

A progressive functional impotence of the limb is thus triggered, to which a parallel limb volume increase also contributes. Last but not least, there is a non negligible risk of malignant transformation of trophic-ulcerative lesions (acanthocarcinoma, lymphangiosarcoma, etc.).

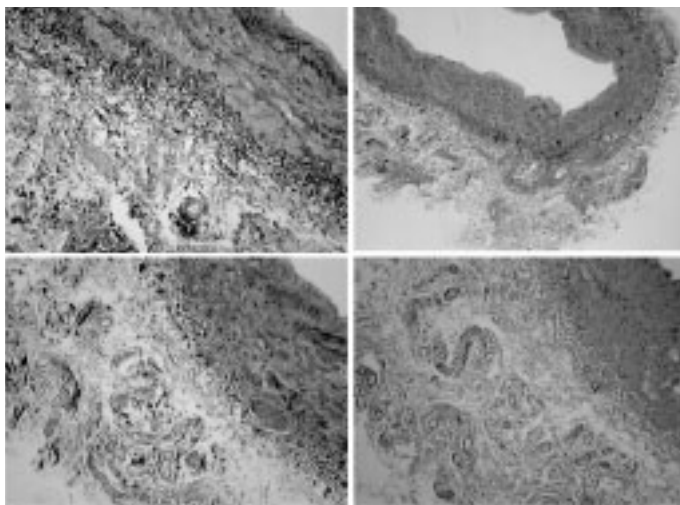


Fig. 1.

Therefore, lymphedema associated with CVI leads to a significantly more severe evolution and poorer prognosis of the clinical picture.

This work aims to demonstrate the following:

- 1) that in chronic phlebolymphe-
dema, vascular imprints are
formed on the surface of the tibia and the fibula;
- 2) that these vascular imprints can be diagnosed on ancient bone
material;
- 3) that vascular casts and imprints found in ancient human remains,
whether associated or not with tibial or fibular periostitis,
are identical in morphology and topographic distribution
to lesions that can be found today in patients affected
by phlebolymphe-
dema;
- 4) to assess the incidence of the lesion in three samples of ancient
medieval populations.

MATERIALS

Seven patients affected by bilateral elephantiasis of the lower limbs have been investigated. They were all long standing IV stage lymphedema cases with significant fibrous tissue component, sclerodermal elephantiasis, and sequelae of recurrent dermatolymphangio-adenites.

A significant sample of skeletons was examined of inhumed or buried individuals from archaeological excavations; in particular, 303 right tibias, 306 left tibias, 165 right fibulas and 165 left fibulas were systematically investigated, with appropriate Paleopathology methods (Table 1). In total 939 bone segments were examined.

The material used for this study was partially taken from the archaeological excavations carried out in Piedmont archaeological sites (Trino Vercellese and Ticineto Alessandrino), and partially from the Church of Puy St Pierre in Briançon (France).

Table 1. Number of bone segments examined in different archeological sites.

	Right Tibia	Left Tibia	Right Fibula	Left Fibula
Trino Vercellese	122	116	95	91
Ticineto	64	63	17	23
Puy St Pierre, Briançon	117	127	53	51
TOTAL	303	306	165	165

Puy St Pierre in Briançon

During conservative restoration works of the Church of Puy St Pierre in Briançon (France) conducted in 1997, ancient human skeletons of adults and subadults (13) were discovered 50 centimeters under the floor. Under the eastern mid portion of the church nave, the bones were stored in a disorderly fashion, without any anatomic connection and they were surfacing from the ground just below the floor; it was probably an ossuary formed by periodic dismantling of the graveyard outside the church (Berge, personal communication).

Only a very rough dating of this material is possible, since this ossuary is assumed to have been used between the 16th and the 18th century.

The material was not buried following any anatomic connection and bone segments are often missing. For this reason, it was not possible to reconstruct the various skeletal districts, nor to attribute the fragments to single individuals. Therefore, in the anthropological study, it was not possible to distinguish between different individuals, but the observation mostly referred to single skeletal elements. A minimum number of 154 adults (left femurs) and 47 subadults (right femur) has been reckoned for this ossuary. The study considered the whole adult population sample (individuals older than 18). All bone surfaces of the tibia and fibula diaphysis that could be assessed by macroscopic morphological analysis have been systematically examined (see Table 1).

Ticineto Alessandrino

This site is located in the area of the municipality of Ticineto Alessandrino. The necropolis was discovered during two excavation campaigns in 1975-76. Excavations were initiated by private individuals, and then carried out by the Archeological Service of Piedmont (14).

The material discovered comes from individual as well as common burials that date back to the 7th and 8th century (15).

A minimum number of 132 adults and 18 subadults was calculated. Male individuals account for 21%, female individuals for 16%, and individuals of undetermined gender for 63% of the adult population sample (Negro Ponzi Mancini, 1999) (Table 1).

Trino Vercellese

The material used for this study was found during excavation campaigns that took place between 1980 and 1991 (Negro Ponzi Mancini, 1999) in the graveyard area north-west of the Church of S. Michele di Trino, as well as inside the church. The material was dated between the 8th-9th and 17th century (15).

A minimum number of 391 adults and 88 subadults was calculated.

Male individuals account for 41.4%, female individuals for 31.7%, and individuals of undetermined gender for 26.9% of the adult population sample (Negro Ponzi Mancini, 1999) (see Table 1).

METHODS

Patients were studied according to the diagnostic therapeutic protocol applied in our centre. In particular, they were all investigated with conventional X-ray as well CT scan.

Before anthropological and paleopathological investigations, all bone remains were cleaned and restored. The material was washed with water and small soft brushes, then placed to dry in a dry environment. Bone segments were first recognized and then restored by gluing together matching fragments with quick setting glue. All materials were then numbered with indelible black ink. During material examination, gender was determined from the morphological features of the skull and the pelvis (16,17).

The biological age at death was determined by looking at the degree of skull synostosis and at the changes of the symphysis pubis surface in adult individuals (17-18). The age of subadult

individuals was assessed by looking at the level of mineralization and dental eruption (19) as well as at the maximum mataphysis length in major long bones of the limbs (20).

Investigations on the lesions caused by subperiosteal bone apposition (commonly called periostitis) were carried out by referring to the Stothers' classification (21), which divides them into four stages of alteration:

- Stage 1 (mild): the bone surface shows small furrows and holes interspaced on a smooth surface;
- Stage 2 (moderate): the bone surface shows deeper furrows and some of its portions look slightly thickened;
- Stage 3 (marked): the bone surface is roughly remodelled with holes and pitting, infection also involves the deeper bone;
- Stage 4 (severe): the volume as well as the profile of the bone are deformed, vascular imprints are visible on the surface, which is a sign of chronic process.

Assessment of pathological lesions with unique Paleopathology methods as well as their description and diagnostic evaluation were conducted according to the criteria applied in this discipline and codified in reference books (22,23,24,25,26,27).

RESULTS

In three out of seven investigated patients, axial tomography imaging showed confined periosteum thickening and vascular imprints to be attributed to perivascular lesions on bilateral tibial diaphysis (Fig. 2).

At paleopathological examination, 56 vascular imprints were observed for all three sites, that were carved up as follows: 18 vascular imprints on right tibias, 10 of them associated with periostitis; 19 vascular imprints on left tibias, 8 of them associated with periostitis; 10 vascular imprints on right fibulas, 3 of them associated with periostitis, and 9 vascular imprints on left fibulas, 3 of them associated with periostitis. (Table 2). In only one case was the lesion bilateral. Individual T15-18 (Ticineto) shows vascular imprints on both tibias, located in different parts: on the right tibia they were found posteriorly in association with grade 1

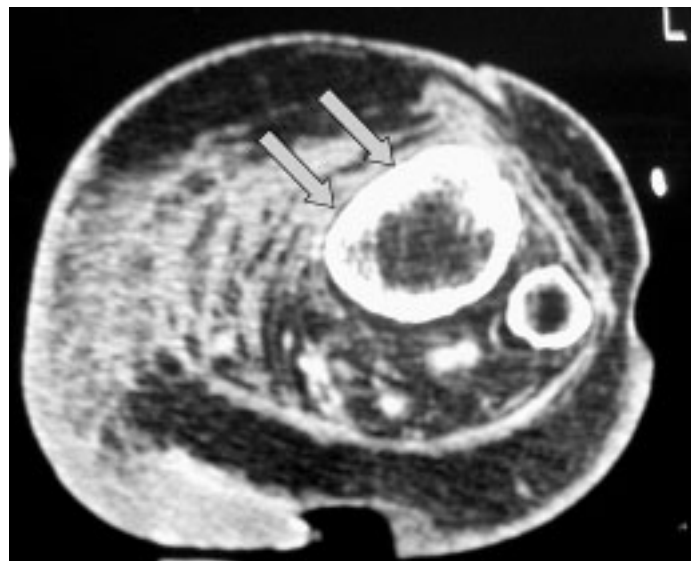


Fig. 2.

Table 2. In tables 2A, 2B and 2C data about vascular casts concerning different sites are reported.

A) Trino Vercellese							
Bone segment	N° observations	N° cases	% cases	N° cases without periostitis	% cases without periostitis	N° cases with periostitis	% cases with periostitis
Right Tibia	122	4	3.28%	0	0%	4	3.28%
Left Tibia	116	5	4.31%	3	2.59%	2	1.72%
Right Fibula	95	4	4.21%	3	3.16%	1	1.05%
Left Fibula	91	4	4.40%	2	2.20%	2	2.20%

B) Ticineto							
Bone segment	N° observations	N° cases	% cases	N° cases without periostitis	% cases without periostitis	N° cases with periostitis	% cases with periostitis
Right Tibia	64	11	17.19%	5	7.81%	6	9.38%
Left Tibia	63	8	12.70%	6	9.52%	2	3.18%
Right Fibula	17	3	17.65%	2	11.76%	1	5.89%
Left Fibula	23	2	8.70%	1	4.35%	1	4.35%

C) Puy St Pierre Briançon							
Bone segment	N° observations	N° cases	% cases	N° cases without periostitis	% cases without periostitis	N° cases with periostitis	% cases with periostitis
Right Tibia	117	3	2.56%	3	2.56%	0	0%
Left Tibia	127	6	4.72%	2	1.57%	4	3.15%
Right Fibula	53	3	5.66%	2	3.77%	1	1.89%
Left Fibula	51	3	5.88%	3	5.88%	0	0%

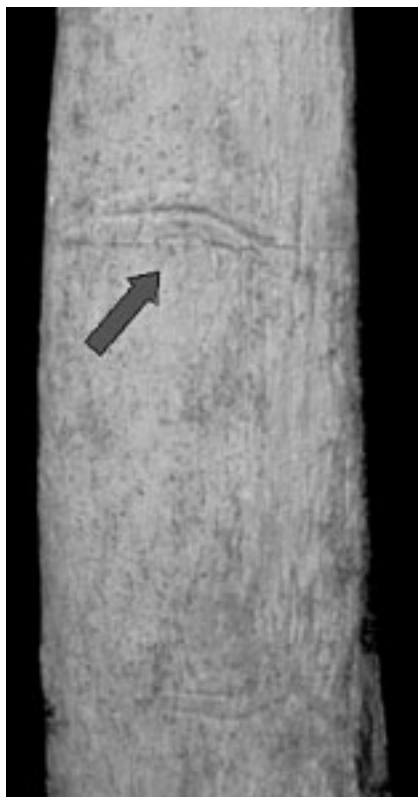


Fig. 3.

periostitis, whereas on the left they were found medially on the bone surface.
 The incidence of vascular imprints is higher for all bone segments in the population segment from Ticineto Alessandrino.
 All in all, 56 lesions were observed, 24 of them associated with periostitis, whereas 32 (57.14 %) featured osteo-periosteal cast only (Figs. 3-5).

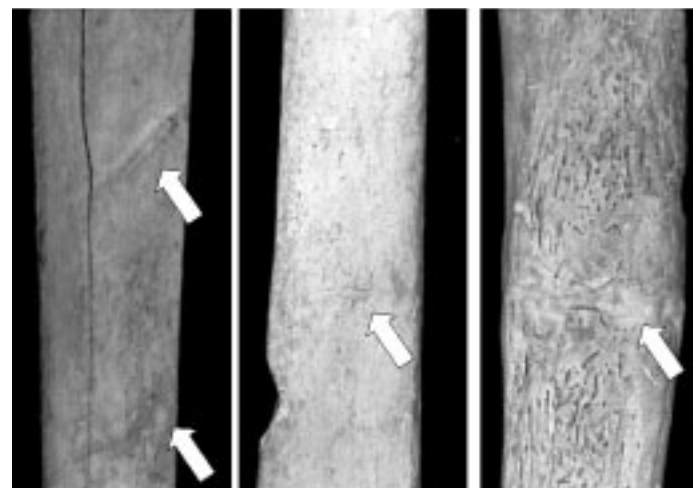


Fig. 4.

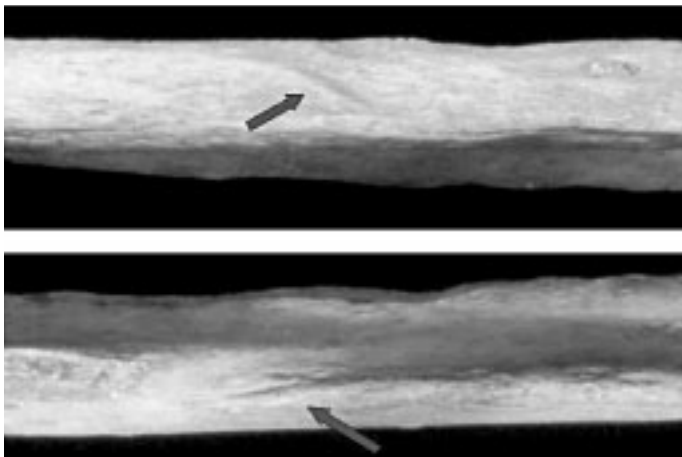


Fig. 5.

Conversely, the overall incidence of periostitis without vascular lesion association was generally low, namely 102 observations out of 939 analyzed bone segments, or 10.86 % (Table 3). With regard to the left and right tibia, lesions were mostly located on the medial bone aspect, whereas with regard to both left and right fibula, lesions were found to occur with a similar incidence on the posterior and lateral aspect of the bone diaphysis (Table 4).

DISCUSSION

Vascular imprints that can be found on the bone surface, whether in association or not with subperiosteal bone reaction - which is generically and imprecisely defined as periostitis - have been debated for some time.

As far as we know, the first detailed observation was made by Per Holck in a 1970 paper (28). He reported about a tibial lesion, accurately described by the author and supported with good iconographic documentation. In the discussion, the author quoted the opinion of Professor Wilhelm Doerr, an authoritative and outstanding pathology expert from Heidelberg, who said that he

Table 4. Tables report data about the prevalence of lesions due to periostitis in the three different places.

Trino Verellese							
	Total observations	1		2		3	
Right Tibia	122	11	9.02%	2	1.64%	0	-
Left Tibia	116	8	6.90%	7	6.03%	0	-
Right Fibula	95	4	4.21%	3	3.16%	0	-
Left Fibula	91	7	7.69%	2	2.20%	0	-

Ticineto							
	Total observations	1		2		3	
Right Tibia	64	15	23.44%	2	13.34%	1	1.56%
Left Tibia	63	19	30.16%	3	4.76%	1	1.59%
Right Fibula	17	1	5.88%	0	-	0	-
Left Fibula	23	1	4.35%	0	-	0	-

Puy St Pierre							
	Total observations	1		2		3	
Right Tibia	117	12	10.26%	4	3.42%	1	0.85%
Left Tibia	127	16	12.60%	2	1.57%	1	0.79%
Right Fibula	53	5	9.43%	0	-	0	-
Left Fibula	51	3	5.88%	0	-	1	1.96%

Table 3. Data and percentage parameters about prevalence of lesions divided according to anatomical site are reported.

	TRINO VERCELLESE						TICINETO						PUY ST PIERRE BRIANÇON					
	Cases without periostitis			Cases with periostitis			Cases without periostitis			Cases with periostitis			Cases without periostitis			Cases with periostitis		
	M	L	P	M	L	P	M	L	P	M	L	P	M	L	P	M	L	P
Right Tibia	25	-	-	50	25	-	55	9	-	27	-	9	100	-	-	-	-	-
Left Tibia	60	-	-	20	-	20	25	-	-	63	12	-	17	17	-	66	-	-
Right Fibula	-	25	50	-	25	-	-	-	67	-	-	33	-	67	-	-	33	-
Left Fibula	-	50	-	-	-	50	-	1	-	50	-	-	-	100	-	-	-	-

Legenda: M = Medial; L = Lateral; P = Posterior.

had made similar observations on living patients affected by chronic edema of the limbs.

On this point, following this authoritative clue, we have tried to gather scientific information and evidence to this pathogenetic assumption. At the same time, we have tried to provide a more accurate lesion description, even with regard to its relations with chronic tibio-fibular periostitis that may be associated with the lesion.

The bone is covered by the fibrous connective layer of the periosteum (just below a very thin coating of mesothelium-like cells) and, under the periosteum, there is the inner layer of the cambium resting on a compact diaphysis bone. Sharpey's fibres run from the periosteum down to the cortical bone; rich in collagen, these fibers anchor the periosteum to the bone. The cambial layer may produce bone with a roughly trabecular pattern. This "sub periosteal bone apposition" becomes a basic and characterizing element of any response to inflammation by the periosteum or by muscular and fascial connective tissues. Rothschild (27), resuming an analysis and classification method first suggested by Resnick and Niwayama in 1988 (29), and used again by Ragsdale in 1993 (30), in a work comparing radiograms and dehydrated bone preparations, identified several types of subperiosteal bone apposition (Table 1). In this way, different types of subperiosteal bone apposition characterize and become diagnostic elements for different diseases.

We believe that a seventh type of bone apposition could be added, namely a perivascular "wax casting" type, which forms a perivascular cast, on which the vessel imprint is left.

The lesion is part of a widespread subperiosteal bone apposition context presenting with several degrees of severity. In general, it features a cast or a furrow which developed not out of bone loss, but rather following bone growth around a stable and chronically inflamed structure. Therefore, the lesion looks like a homogeneous cast (apparently a negative imprint into the tissue), which features the mould of a vessel. Generally, one third of the vessel circumference is included in the newly grown tissue.

56 lesions were observed in total. 32 of them feature single or multiple lesions without periosteal reaction in the involved bone segment. Conversely, a widespread subperiosteal bone apposition, acting as a substrate to the described lesion, is present only in 24 cases. This observation, on the one hand, seems to prove that the lesion is not to be considered as a simple reaction to chronic periostitis, but rather as an expression of chronic venous disease. In this light, subperiosteal bone lesions are most likely to be the consequence and hence the sign of longstanding disease.

Therefore, there are likely to be two different, often mutually related, pictures of lymphedema with phlebolymphedema in cases without periostitis association, and of chronic fibroplastic and osteoplastic periostitis with inflammatory vasculopathy in cases where vascular imprints are associated with periostitis.

On the other hand, in the sample of investigated individuals, there are several cases of "periostitis" without vascular imprints. This would once again confirm the extremely high variety of pictures and root causes of periostitis (4) that escape any simplistic definition, although all of them result from trauma.

Through a retrospective study of current patients with phlebolymphedema, axial tomography scans could be assessed again, thus proving the presence of similar lesions. Bone imprints

of vascular origin have thus always proved to be associated with chronic vessel inflammation and to be a consequence of this condition. Finally, also the course of disease and patient staging have shown that vascular lesions of various severity are in any case to be correlated with chronic lymphedema.

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48th ANNUAL MEETING OF THE GERMANY SOCIETY OF PHLEBOLOGY

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2006 - 15-18 November, Rome (ITA)

XVIII CONGRESSO NAZIONALE SIAPAV 2006 GIORNATE DI ANGIOLOGIA

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XXXII g.e.l.-Congress

Hinterzarten/Germany

May 12-14, 2006

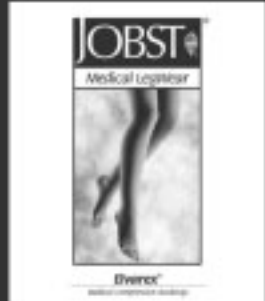
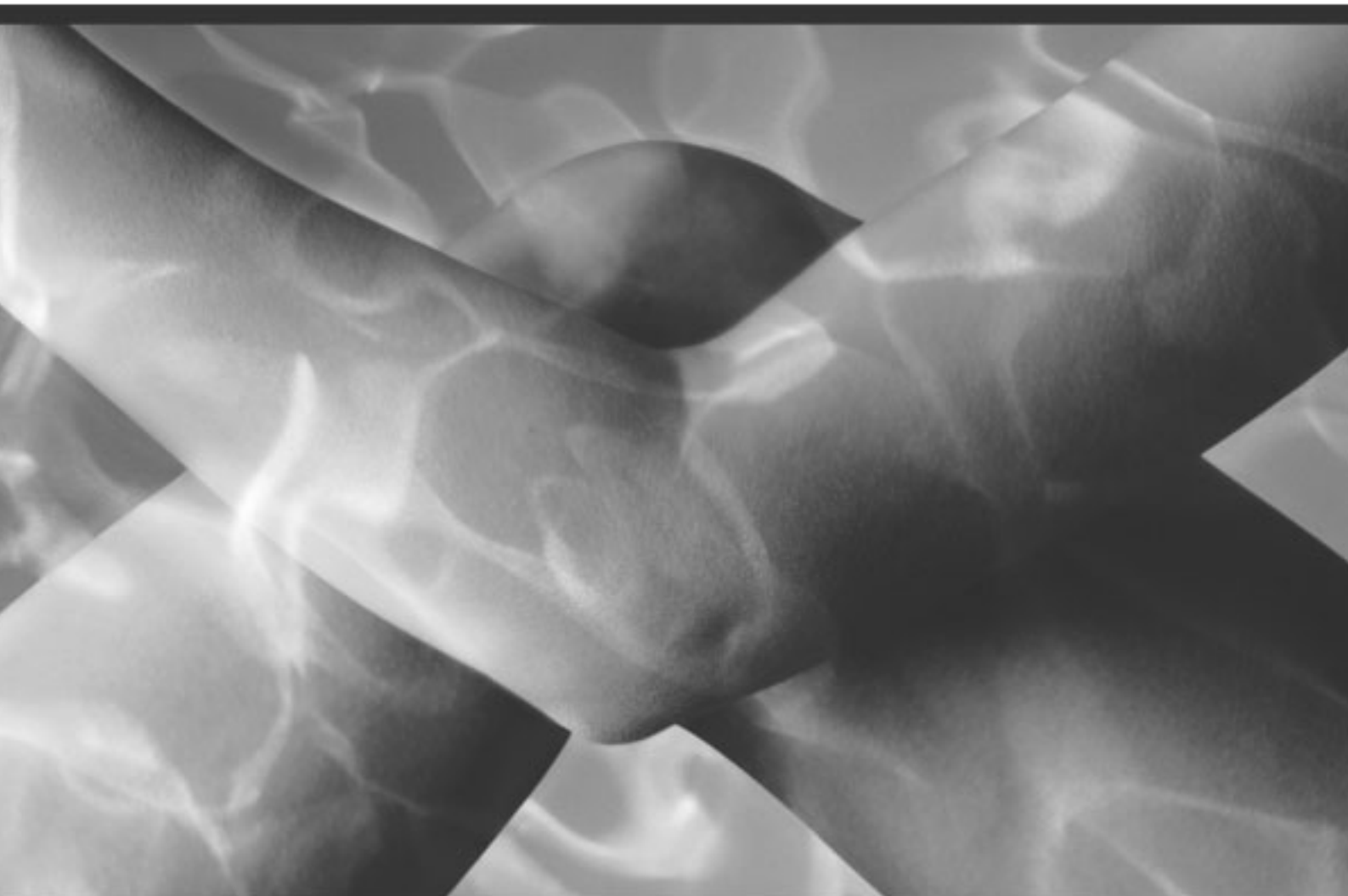


Congress language: English
with simultaneous translation
(english/german)

Friday, May 12, 2006	
15.00-18.00	Executive Committee meeting of the ISL <i>Földiclinic, conference room</i>
20.00	Dinners party: Members of the Executive Committees of the ISL, the g.e.l. and the GDL <i>Parkhotel Adler</i>
Saturday, May 13, 2006 <i>Kurhaus Hinterzarten</i>	
08.00-08.30	Greetings E. Földi, P. Bourgeois
08.30-10.45	SESSION I The sense of staging lymphedema: Which criteria are valid? <i>Chairmen:</i> M. Földi, U. Brunner, A. Bollinger, W. Olszewski <i>Statements</i> <i>Clinical picture:</i> E. Földi, P. Cluzan, T. Ryan <i>Histology:</i> E. Kaiserling, J. Daroczy, O. Eliska, W. Marsch <i>Imaging techniques:</i> A. Pecking, C. Lohrmann, A. Behar, P. Bourgeois, M. Marshall <i>Discussion</i> <i>Summary:</i> E. Földi
10.45-11.00	Coffee break
11.00-12.30	SESSION II Prevention of lymphedema <i>Chairmen:</i> M. Mortimer, S. Michelini, O. Leduc, G. Thibaut <i>Statements:</i> A. Pissas, K.P. Martin, B. Amann-Vesti, S. Albert, D. Corda <i>Discussion</i> <i>Summary:</i> P. Mortimer
12.30-13.30	Lunch / lunch-meeting GDL, Kurhaus Hinterzarten

13.30-15.15	<p>SESSION III</p> <p>Conservative treatment of lymphedema; long term results</p> <p><i>Chairmen:</i> A. Leduc, K. Johansson, M. Andrade, M. Jünger</p> <p><i>Statements:</i> O. Leduc, S. Michelini, U. Franzeck, M. Oberlin, G. Thibaut, K. Johansson, R. Strößenreuther</p> <p>Discussion</p> <p><i>Summary:</i> R. Baumeister</p>
15.15-15.30	Coffee break
15.30-17.15	<p>SESSION IV</p> <p>Reconstructive surgical treatment of lymphedema</p> <p><i>Chairmen:</i> C. Campisi, C. Papendieck, R. Baumeister</p> <p><i>Statements:</i> F. Boccardo, H. Brorson, R. Baumeister</p> <p>Discussion</p> <p><i>Summary:</i> O. Leduc</p>
17.30-18.30	Executive Committee meeting g.e.I., Parkhotel Adler
20.00	Gala Dinner, Parkhotel Adler
	Workshop, Földiklinik Hinterzarten
09.00-12.00	Compression therapy of lymphedema. What is new?
12.00-14.00	Lunch
14.00-17.00	<p>Practice of compression bandaging. What kind of compression garment?</p> <p><i>Lecturer:</i> H. v. Zimmermann, P. Asmussen, G. Klose, H. Thoma, C. Moffat, B. O'Kane</p>
<p>Sunday, May 14, 2006</p> <p><i>Kurhaus Hinterzarten</i></p>	
08.00-09.15	<p>SESSION V</p> <p>Additive surgical treatment of lymphedema</p> <p><i>Chairmen:</i> B. Stark, C. Papendieck</p> <p><i>Statements:</i> G. Felmerer, E. Lang, N. Liu</p> <p>Discussion</p> <p><i>Summary:</i> W. Olszewski</p>
09.15-11.00	<p>SESSION VI</p> <p>Basic Lymphology - What is new?</p> <p><i>Chairmen:</i> W. Olszewski, M. Witte, N. Grande</p> <p><i>Statements:</i> J. Wilting, E. Fulcheri, O. Eliska, M. Ohkuma, M. Bernas</p> <p><i>Summary:</i> M. Witte</p>
11.00-11.15	Coffee break
11.15-12.45	<p>SESSION VII</p> <p>European Consensus: Diagnostic and therapy of lymphedema</p> <p><i>Round table/statements:</i> A. Pecking, M. Földi, J. Belgrado, C. Campisi, R. Baumeister, G. Thibaut, O. Leduc, S. Michelini</p> <p><i>Summary:</i> M. Koller</p>
12.45-13.30	General Assembly of g.e.I., Kurhaus Hinterzarten

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