ISSN 0778-5569

OFFICIAL ORGAN
OF THE

EUROPEAN GROUP OF LYMPHOLOGY
LATIN-MEDITERRANEAN CHAPTER
OF ISL
SOCIETÀ ITALIANA DI LINFANGIOLOGIA
CZECH SOCIETY OF LYMPHOLOGY
ROMANIAN SOCIETY OF LYMPHOLOGY

THE EUROPEAN JOURNAL OF lymphology

and related problems

VOLUME 16 • No. 49 • 2006

INDEXED IN EXCERPTA MEDICA

SUMMARY

CLINICAL SCIENCES

Original articles

– Translational lymphology and the Földiklinik
Marlys H. Witte, M.D. p. 1

– Imaging findings in pulmonary lymphangiectasia
Carlo Bellini, M.D., P.H.D.; Francesco Boccardo, M.D., P.H.D.; Corradino Campisi, M.D.; Eugenio Bonioli, M.D. p. 10

– Demonstration of Flowave's effectiveness through lymphoscintigraphy
Maurizio Ricci, Simona Paladini. p. 14

– Early or late diagnosis of Lymphedema in our Lymphedema unit
Isabel Forner-Cordero, Raquel Navarro-Monsoliu, Jose Muñoz-Langa, Pilar Rel-Monzó p. 19

– Role of lymphoscintigraphy in the indications to microsurgical treatment of peripheral lymphedemas
Francesco Boccardo, Carlo Bellini, Corrado Campisi, Costantino Eretta, D. Pertile, Emanuela Benatti, Mirko Campisi, Giuseppina Talamo, Alberto Macciò, Corradino Campisi p. 24

Calendar

PRI-MED MID-ATLANTIC - NOVEMBER 30 / DECEMBER 2, 2006 - BALTIMORE (MARYLAND)

SOCIETÀ ITALIANA DI FLEBOLOGIA - 5th MEDITERRANEAN CONGRESS OF PHLEBOLOGY - 28 APRIL / 1 MAY, 2007 PORTO HELY (GREECE)

XXXIII CONGRESS OF EUROPEAN GROUP OF LYMPHOLOGY - MAY 12-13, 2007, PRAGUE (CZECH REPUBLIC)

CONGRESO ARGENTINO DE FLEBOLOGIA - 23 A 25 DE MAYO DEL 2007 - TUCUMÁN (ARGENTINA)

INTERNATIONAL UNION OF PHLEBOLOGY (UIP) - WORLD CONGRESS CHAPTER MEETING - 18 AL 20 DE JUNIO DE 2007 KYOTO (JAPAN)

SOCIETÀ ITALIANA DI FLEBOLINFOLOGIA (SIFL) - V CONGRESSO INTERNAZIONALE DI PHLEBOLOGY - 1-4 LUGLIO 2007 CORFÙ

21st INTERNATIONAL CONGRESS OF LYMPHOLOGY - SEPTEMBER 26-30, 2007, SHANGHAI (CHINA)

UNION INTERNATIONALE DE PHLEBOLOGIE (UIP 50) - 16° CONGRESSO MONDIALE - AUGUST 31 / SEPTEMBER 2, 2009 GRIMALDI FORUM - PRINCIPATO DI MONACO p. 29

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 16 • No. 49 • 2006
INDEXED IN EXCERPTA MEDICA

TRANSLATIONAL LYMPHOLOGY AND THE FÖLDIKLINIK

MARLYS H. WITTE, M.D.
Department of Surgery, University of Arizona, Tucson, Arizona USA

Corresponding author: Marlys H. Witte, MD
Professor of Surgery
University of Arizona
1501 N. Campbell Ave., Room 4406
P.O. Box 245200
Tucson, Arizona 85724-5200
e-mail: lymph@u.arizona.edu

ABSTRACT

The opening of the new wing of the FöldiKlinik in Hinterzarten, Germany, in October 2004, afforded the opportunity to reflect on the recent advances in molecular lymphology and their potential impact on basic research and clinical lymphology. We are now in a position both to expand our delineation of the lymphatic "phenotype" (particularly through non-invasive, multimodal imaging) as well as pinpoint the lymphvascular genotype and its structural and functional expression in patients with lymphedema, lymphangiodysplasias, and other lymphatic system disease. New therapeutic approaches, in addition to current non-operative and operative options, are on the horizon, including gene therapy, lymphangioinhibitory and stimulatory drugs, stem cell therapy, and tissue engineering. As we translate these advances from bench to bedside and clinic, more than ever global collaborations and advanced communication technologies (e.g.,TeleLymphology based at the University of Arizona Health Sciences Center) are needed to carry out optimal testing and evaluation of these alternatives for therapeutic benefit vs. risk and also to assure the widespread availability of effective modalities throughout the world community. The newly expanded FöldiKlinik will be a premier hub in this effort.

KEY WORDS: lymphedema, lymphology, lymphvascular phenotype, lymphvascular genomics, translational medicine, telemedicine, FöldiKlinik.

BACKGROUND

What an exciting moment and great honor it was for me to be present for the celebration of the opening of the new wing of the FöldiKlink! In the United States, there's a movement afoot called "translational medicine," encouraging physicians and basic scientists to come together to expedite the movement of basic science advances from the "bench to the trench," i.e., from the laboratory to the bedside/clinic and community. Whereas translation may be a new movement for some specialties, it's nothing new for lymphology and lymphologists. Since before the official founding of the International Society of Lymphology in 1966 and the introduction of the new word "lymphology" for this discipline by visionary Swiss radiologist Alois Rüttimann and his co-founders, we have recognized that translational biologists need to work alongside translational physicians and transcend the barriers of language, geography, and specialization. There's hardly a lymphologist, exemplified by pioneers such as Ernest Starling exploring the physiologic principles governing lymph formation and edema or Florence Sabin meticulously dissecting the lymphatic development of the human embryo, who wasn't looking, either as basic scientists to implications of their findings for clinical practice, or, on the other hand, astute clinicians, like British surgeon John Kinmonth seeking out basic scientists for better explanations of the inheritance patterns and dysfunctional lymphographic images he was seeing for the first time in patients with lymphedema-angiodysplasia syndromes including the familiar hereditary forms.

Since the 1960's, the Földis, Michael or Ethel, one or the other or both together, have framed the boundaries of lymphology with their ideas, experiments, teachings, and clinical practice. Publication of the landmark tome by the Hungarian team of Rusznyak, Földi and Szabo, in 1960 (Rusznyák et al., 1967) defined the field, describing lymphostatic disorders, and presenting some commonalities, themes, and theories of their pathophysiology based on experimental study and clinical observation. This was the moment, in essence, lymphology was introduced to the global scientific community. As lymphographers brought the hidden lymphatic vasculature and lymph nodes into view, displaying vivid living images of lymphatic disorders, the multidisciplinary International Society of Lymphology took shape and purpose to provide an international forum and dispel the notion that the lymphatic system was simply "lymph nodes held together by strings" (Lymphology, 1967-2006; Progress in Lymphology, 1967-2006). Again, Michael Földi was there for the celebration and interchange and soon, Ethel joined him, first as a basic scientist exploring the pathophysiology and potential treatments of experimental lymphostatic diseases, and then she moved from the laboratory into the clinic to join Michael and apply, for the first time, these pathophysiologic concepts to diseases which had previously been approached in a disorderly, haphazard fashion. Their clinic and then a full-fledged hospital gradually evolved into the world's premier medical institution devoted exclusively to lymphology with associated educational and certification programs to ensure a coordinated clinical care team of multidisciplinary experts for these patients. Then in 1994 in Hinterzarten, Germany, the Földis, in consultation with the ISL

Executive Committee, created the framework for the first international consensus statement on lymphedema (ISL Executive Committee, 1995). The publication and dissemination of this document stimulated a vigorous dialogue about the optimal approach to diagnosis and treatment of lymphedema and established international guidelines albeit as a work in progress. A few years later, in the mid- and late 1990's, molecular lymphology exploded on the scene with major discoveries in genomics, proteomics, lymphangiogenesis, immunohistochemistry, and systems biology, bringing with them the potential for revolutionary advances in clinical lymphology (reviewed in Witte et al., 2003).

So, what about lymphology's "next future" (to borrow this quaintly English expression from my Italian lymphologic colleagues)? The expression implies that there is a future now, and then there is a "next future", one that I'd like to highlight for the next few minutes. The opening of the new wing of the FöldiKlinik provided an opportune moment to reflect and also to set the stage for a prototype collaborative network of lymphology centers in this next future of clinical care, research, and education, i.e., translational lymphology, so that advances at the laboratory bench can move rapidly yet judiciously to the bedside and spread throughout the global community.

LYMPHVASCULAR PHENOTYPES

The clinical images depicted in Fig. 1 can be viewed as the spectrum of lymphvascular "phenotypes" exhibited by patients with congenital and acquired disturbances of the lymphatic system, i.e., disorders of the "blood-lymph loop." Epigenetics – the interaction of environmental influences with the genetic makeup (the genotype) of these individuals has produced the clinical manifestations of underlying pathologic processes. The phenotypes may be secondary to diseases which occur after birth, or they may arise in fetal life and even be "embryonic lethal" and never seen. But what we do see are the edematous states, scarring disorders, nutritional depletion, e.g., with chylous reflux syndromes, immunodeficiency/dysregulation syndromes and disorders of angiogenesis, often mixed hemangiogenesis-lymphangiogenesis (Fig. 1).

First, a moment's pause to reflect on some of the ignorance relating to these conditions, what we don't know, not necessarily the most important questions, but curious ones, about the edemas: Why does it affect only one side if the condition is hereditary and due to a single mutated gene? Why only one leg, and why the leg, and much less frequently the arms? Why do some lymphatic systems fail with this lymphatic dysplasia, and others display a similar abnormality on lymphscintigraphy but have no edema? The underlying problems need to be delineated. If family members are labeled "unaffected" in a whole genome search and gene linkage study based on whether or not lymphedema is manifest rather than whether disordered lymphangiogenesis is present, gene linkage analysis will be flawed and gene mutations difficult to pinpoint.

Regarding the scarring diseases: What about chronic lymphedema leads to fibrosis and fatty tissue accumulation? Are there drugs that would dissolve the scar, or better yet, prevent scarring? What measures might prevent the initial lymphostasis, or keep it from persisting?

Regarding chylous reflux syndromes: What events lead to megalymphatics and absent or incompetent lymphatic valves? What molecular mechanisms lie behind valve formation? Which gene(s) and protein cascade(s) underlie intrinsic contractility of the lymphatics and pacemaker activity?

Regarding immunodeficiency/immunodysregulation and unhinging of the "blood-lymph loop" in lymphostatic disorders: The lymphatic system is not simply a distinctive vasculature (lymphatic vessels), nor its fluid content (lymph), nor its lymph nodes, nor its working and trafficking immune cells (lymphocytes). The lymphatic system operates in continuity and encompasses the immune system. It is not "lymphoid"; "-oid" implies something false, phony, or faux about it but it's the real thing. How does the system and its components work together (and with other organ systems), and how better might these complex system dynamics be observed in vivo? Can we actually see the trafficking lymphocytes entering and exiting the circulation, the lymphatic trunks pumping or failing, and the molecular signals flashing distinctive colors in vivo in patients with lymphatic diseases? And will we be able to watch on remote

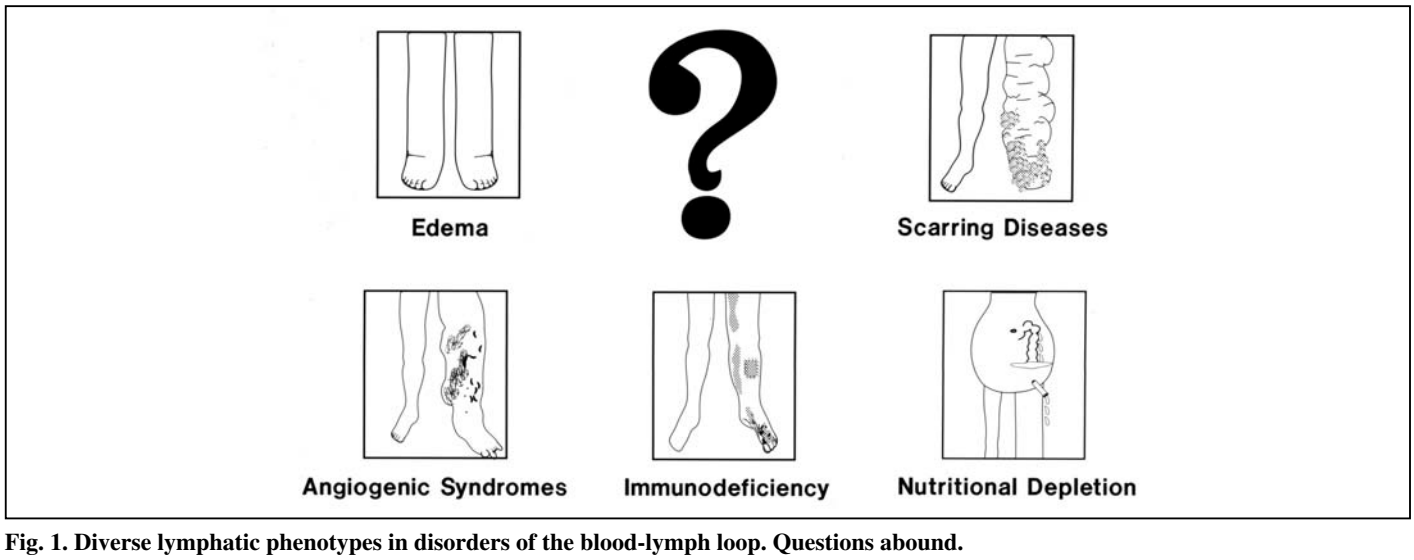


Fig. 1. Diverse lymphatic phenotypes in disorders of the blood-lymph loop. Questions abound.

microsensor printouts as voluminous data is generated on the effects of designer drugs or as robotic surgeons normalize the pathologic process?

Regarding the angiogenic syndromes: What about lymphostasis promotes overgrowth of multiple cell types leading to fibrous scar, fat deposits, and also proliferation of blood vessels and lymphatics and how does this pathologic process relate to angiomas and vascular birthmark syndromes? When and how does lymphangiogenesis escape control and become frankly neoplastic? And how soon, as Cristobal Papendieck implores us, will anti-angiotumorigenesis agents be available to prevent or reverse the process and involute those disfiguring lymphangiomas and hemangiomas with a non-toxic pill rather than an extensive endangering operation?

Lymphologists tend to be a pure breed but often with other pastimes. Though respectful of the venous system and its links to the lymphatic system, they are usually neither "lymphophlebologists" nor "phlebolymphologists," though occasionally lymphologist- something else (e.g., -radiologist, surgeon, dermatologist, or internist, etc.). Lymphedema is an external or internal manifestation of excess tissue fluid accumulation resulting from low-output lymph circulatory failure. But it is not a disease entity. The disease is the underlying lymphvascular system abnormality which often but not always culminates in lymphedema. Fifty years ago the expression "cardiogenic edema" was still commonly used signifying that we didn't know the underlying heart diseases well enough on a pathophysiological or molecular level to define the cause. Now we can often pinpoint the origin of the heart disease and the specific cause of the heart failure and thereby the pathomechanism of "cardiogenic edema," and so the latter term has been abandoned. In the same way, we are beginning to talk about the underlying lymphatic disturbance, even the causative gene mutations for lymphatic maldevelopment and "lymphologenic edema." For example, the transcription factor FOXC2 is mutated in lymphedema-diastichaisis (LD) syndrome (Fang et al., 2000), producing a double row of eyelashes, and a hyperplastic refluxing lymphatic system, first described by lymphologist-surgeon John Kinmonth on conventional lymphogram (1972) in families he had collected together. Genetically engineered mice with Foxc2 deficiency mirror closely the phenotype of human LD (Kriederman et al., 2003). Although we haven't deciphered how exactly this gene or gene mutation works, we can delineate the specific LD phenotype satisfactorily in several ways – on general clinical examination, conventional lymphography, and with non-invasive lymphscintigraphy. During the next few years, there will be new and better ways with dual modality dynamic techniques, viewing images superimposed on each other, to anatomically define lymphatic disorders; we will be able to see smaller and smaller things with higher resolution, better differentiated within non-homogeneous tissues and even pick up molecular signals from surrogate markers of disease and provide molecular snapshots of specific cell populations as they attack, attract, or defend during disease processes and treatment efforts. Distinctive subpopulations of lymphocytes will be separable from each other, and tumor areas shown forming near initial lymphatics in microarrays of laser-dissected lymph nodes. A whole new world of "nanolymphology" is on the verge of unfolding.

Thus, advanced imaging is the key to "phenotype" more deeply – to go beyond the swollen foot and Stemmer sign – to uncover the cause and the dynamic molecular and cellular events of the underlying pathophysiologic processes. Multimodal imaging à la Charles Witte (Table 1; Witte and Witte, 2000) envisioned these combinations of state-of-the-art imaging technologies to define the clinical problem as a rational basis for a treatment plan. But now we would not have to get an MRI in this room and a lymphangioscintigram (LAS) a week later in another department somewhere else. Images increasingly more molecular and cell-based will be generated in combination all at the same time so that they can be juxtaposed and analyzed rapidly in much more detail and dynamically, functionally, and continuously over time.

Table 1. Imaging Algorithm for Evaluation of Lymphedema-Angiodysplasia Syndromes.

Modality	A-V	Venous	Lymphedema	Lymph-angioma	Chylous	Lymphangio-myomatosis	KT-S	Lipedema
MRI		++++	++	++++	++++		++++	++++
MRA		+++		+++			++++	
LAS			++++	+++	++++		++++	++++
CA	++++							
CL					++			
CT			++			++++		
PLAIN		+					++	
US	+++	+++	++ (Filariais)					
CV		+++						

Scale of + (low) to ++++ (high) suggests relative usefulness of test in clinical setting; * = with gadolinium; ** = with fat suppression (STIRS).

MRI = magnetic resonance imaging; MRA = magnetic resonance arteriography; LAS = whole body lymphangioscintigraphy; CA = conventional arteriography; CL = conventional lymphography; CT = computed tomography; PLAIN = radiographs; US = ultrasonography; CV = conventional venography; A-V = arteriovenous malformations; KT(-S) = Klippel-Trenaunay (-Servelle) syndrome. Modified from Witte et al., 2000.

LYMPHVASCULAR GENOTYPES

Moving from lymphatic phenotyping to lymphatic genotyping, forward and reverse techniques are used to explore hereditary syndromes (Fig. 2). In September 2005, in Salvador, Brazil at the 20th International Congress of Lymphology, we (Peter Mortimer, Marlys Witte, and Michael Bernas) featured a one-hour course on "Genetics for Lymphologists." We began with a patient presenting with lymphedema and a positive family history, outlined an approach to clinical assessment, described procedures for obtaining DNA, searched the whole genome for linkage of affected individuals to specific chromosomal loci, sequence candidate genes for mutations in the narrowly defined chromosomal region, and then returned to the clinic to inform and counsel the patient and family regarding the genetic findings and their implications for management now and in the future. Reverse genetics starts with the patient, zeroes in on the likely location of gene mutations and then cones down on smaller and smaller regions of the DNA code through marker analysis and even further, as in our collaborations with David Duggan of the Translational Genomics Corporation (TGen) in Phoenix, Arizona, with 10K or 100K "SNPs," single nucleotide polymorphism analysis. This approach allows rapid screening of the entire genome in only a few days, not weeks or months, using sophisticated software for complex bioinformatics and statistical analysis to pinpoint the affected chromosome region and accelerate the discovery of specific malfunctioning genes.

Forward genetics, on the other hand, identifies a likely candidate gene in advance, e.g., a lymphatic growth factor or its receptor, or another protein associated with lymphatic structure or function. Then the gene is either 'knocked out' in a mouse, allowing study of either one copy of that gene, (half of the complement), both copies (wild type), or none (null), or the opposite, the gene can be overexpressed. These mice are then studied to delineate the resultant lymphatic and general clinical phenotype. These are powerful genomic technologies spun off from the Human Genome Project that are advancing by the day. Equipment that costs a million dollars is obsolete six months later, because it's about a tenth as fast as new equipment that's coming out, giving much more information, faster, and more economically.
Genes, growth factor ligands and endothelial receptors involved in the development of blood vessels have been extensively studied since the early 1990's. The process of vessel formation de novo from angioblasts (vasculogenesis), and progressive vessel evolution and remodeling into the branched vascular tree of arteries, veins and capillaries follows through vessel sprouting and pruning. Lymphatic development probably proceeds through an analogous process also involving the vascular endothelial growth factor (VEGF) and angiopoeitin families of growth factors and receptors. Whereas the preponderance of current opinion favors the origin of lymphatics from veins, the evidence is not so clear. As a lymphologist, I have difficulty accepting in my heart (or thoracic duct) and intellectually, that the entire lymphatic system is merely a "bud" off the central veins (centrifugal theory). Indeed, Jörg Wilting and his team in Goettingen are mounting molecular evidence (supported by earlier injection studies and electron microscopic examination) that the lymphatic system – vessels and nodes – at least in part does arise independently in the periphery and within the viscera, but not from sprouts of the central or even regional venous system. Within the next few years, this controversy (centripetal vs centrifugal theory) may be resolved and the lymphatic system shown, at least in part, to be primary as suggested in his lead article in the September 2004 issue of Lymphology, "Is the lymphatic system secondary or primary?" (Wilting et al., 2004) and supported by evidence collected by others before and since.

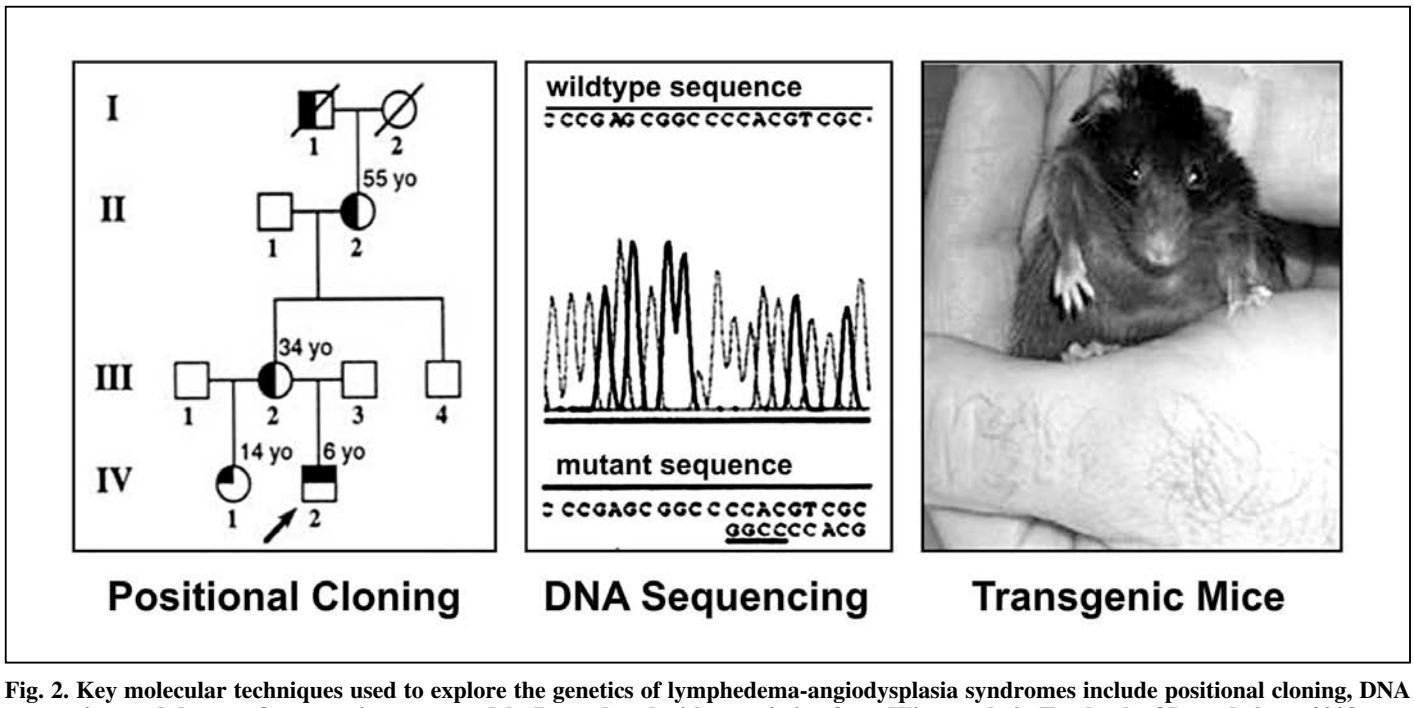


Fig. 2. Key molecular techniques used to explore the genetics of lymphedema-angiodysplasia syndromes include positional cloning, DNA sequencing, and the use of transgenic mouse models. Reproduced with permission from Witte et al., in Textbook of Lymphology, 2003.

The specific growth factors, receptors, and transcription factors that have been described to influence lymphatic growth and development are shown in Table 2 (modified from Witte et al., 2003). Some are not unexpected, like the members of the VEGF and angiopoeitin families of growth factors and their corresponding endothelial receptors. But there are also transcription factors, such as FOXC2, SOX18, and PROX1. Who would have imagined that the fruit fly "forkhead" gene Foxc2 would have anything to do with the human lymphatic system and furthermore cause, when defective, the very specific clinical syndrome of lymphedema-diastichaisis? There will be more surprises, and any all-encompassing scheme of lymphatic development is likely to be extensively revisited over the next few years as more discoveries are made and the interacting pathways and feedback loops prove to be more complex than first envisioned.

The Foxc2 haploinsufficient (+/-) mouse (Kriederman et al., 2003) with just one allele knocked out phenotypically resembles the patient I encountered in my first visit in the early days of the FöldiKlinik several decades ago. She had a double row of eyelashes and pubertal onset lymphedema and a family history of the condition. The mice, like the lymphedema-distichiasis patients with FOXC2 mutations (Fang et al., 2000), invariably show a double row of eyelashes and often exhibit a hyperplastic refluxing lymphatic system resembling Kinmonth's distinctive lymphograms (1972) with occasional limb edema along with a variety of other ocular abnormalities like their human counterparts. A smaller number of affected humans exhibit cleft palate and tetralogy of Fallot. When Foxc2 is completely knocked out – homozygous null mouse (-/-) – they die before birth displaying both cleft palate and combined aortic arch and cardiac interventricular septal defects, closely resembling tetralogy of Fallot (Iida et al., 1997); they also exhibit defective lymphatic valvular formation (Petrova et al., 2004) as documented earlier by extensive visceral lymph reflux seen in the Foxc2 haploinsufficient mouse (Kriederman et al., 2003). Curiously, the Foxc2 overexpressing mouse (with an adipocyte promoter) also exhibits lymphatic system hyperplasia like the haploinsufficient mouse but without distichiasis or other ocular abnormalities (Noon et al., 2006) suggesting that Foxc2 gene dose imbalance may be involved in the distinctive lymphatic phenotype.

On the other hand, the angiopoietin-2 transgenic mouse phenotypically resembles a variety of human lymphedema syndromes. When the gene for vascular growth factor angiopoeitin 2, known to be involved in the modeling and remodeling of blood vessels, has been totally knocked out (-/-), we have found no lymphatics or lymph nodes (or only very rudimentary ones) in the lower limbs, and the upper limb and cervical lymphatic system is also hypoplastic or aplastic (Gale et al., 2002). Lymphedema of the lower limbs and chylous ascites and chylothorax are typically present. But no reported human lymphedema syndromes have been associated with angiopoietin-2 mutations, i.e., this is a genetically engineered "mouse syndrome" looking for a human genetic counterpart.

When the human lymphvascular genome map (Fig. 3), as we know it today, is laid out with the chromosomes lined up in pairs as a karyotype, potential genes or loci pertaining to lymphatic development are found on most chromosomes. Beginning in 1998, three publications (Ferrell et al., 1998; Witte et al., 1998; Evans et al., 1999) including one in *Lymphology,* linked a form of Milroy syndrome to the distal arm (q34-35) of chromosome 5, and multiple mutations in VEGFR3, the receptor for VEGF-C, have been reported (Karkkainen, 2000). Subsequently, two other lymphangiogenesis genes have been identified in other hereditary lymphedema syndromes. On chromosome 16, is the causative FOXC2 gene for lymphedema distichiasis discovered in a University of Arizona-University of Michigan collaboration (Fang et al., 2000). On chromosome 20 is the gene for transcription factor SOX18 associated with lymphedema-hypotrichosis-telangiectasia syndrome (Irrthum et al., 2003). In addition, other chromosome locations but not the specific genes have been linked, that is, we know where the genes are, but not which ones and how they are altered.

Chromosomal aneuploidy – extra or fewer chromosomes than the normal diploid complement of 46 + X,X or X,Y – are commonly associated with lymphatic maldevelopment (Northup et al., 2003). Aneuploid embryos often die early in fetal life even before lymphatic development takes place, but some such as trisomy 21 and Klinefelter syndrome can even reach adulthood.

Rearrangements or "chromosomal mutations," where a piece of chromosome is deleted, added, or translocated, involve groups of

Table 2. Vascular Growth Factors, Receptors, and Transcription Factors in Vascular, Including Lymphatic, Development

	Vascular Growth Factors								
	PIGF	VEGF-A	VEGF-B	***VEGF-C****	***VEGF-D****	VEGF-E	***Ang-1****	***Ang-2****	***Ephrin-B2****
VEGFR-1/Flt-1	◆	◆	◆						
VEGFR-2/Flk-1		◆		◆		◆			
VEGFR-3/Flt-4*				◆	◆				
Neuropilin-1	◆	◆	◆			◆			
Neuropilin-2*	◆	◆		◆					
Tie-1							◆		
Tie-2*							◆	◆	
Eph-B4									◆

◆ Corresponding growth factor-receptor combinations. *Implicated specifically in lymphatic development (bold and italic text).

genes that get moved, dropped off or duplicated. These also can manifest as lymphatic maldevelopment with limb or more generalized lymphedema, cystic hygromas, chylous effusions and reflux syndromes, and fetal hydrops. Furthermore, when the known chromosomal locations for other linked but unidentified genes are added to those for the already identified genes (Fig. 3), and the genes for the proteins (e.g., VEGF-C and the angiopoietins 1 and 2) already known to be involved in lymphatic development, we see an even more extensive map of lymphatic system-related chromosomes.

Thus, the vast majority of chromosomes are potentially involved in lymphatic development and possibly implicated in lymphatic maldevelopment syndromes. No such comprehensive picture has been proposed for syndromes of blood vascular maldevelopment. The phenotypic features of the nearly forty hereditary familial lymphedema syndromes – autosomal dominant, autosomal recessive and sex-linked are associated with other organ system involvements (Fig. 4; Northup et al., 2003). The commonest association is with head and neck abnormalities, stressing the importance of closely examining children with peripheral lymphedema for dysmorphic features and eye abnormalities as well as cardiovascular and gastrointestinal anomalies.
In addition, there are many other sporadic non-familial lymphedema syndromes, some such as Klippel Trenauney Syndrome combined with blood vessel abnormalities. The atlas in the December 2003 Lymphology (Northup et al., 2003) covers a hundred years of literature including photos and other images to illustrate the spectrum of dysmorphic phenotypes. There remain many collected families who exhibit neither known mutated genes nor linkage to reported loci. And therefore there is a long way to go to find the constellation of genes, proteins and processes involved in lymphatic disease and ultimately to find ways to prevent or treat the various conditions.

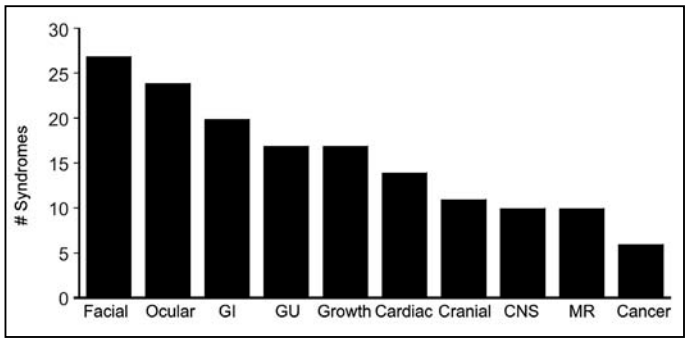


Fig. 4. Phenotypic abnormalities (organ system involvement) commonly associated with the 40 OMIM-listed hereditary LE-AD syndromes. GI = gastrointestinal, GU = genitalurinary, MR = mental retardation. Reproduced with permission from Northup et al, *Lymphology,* 2003.

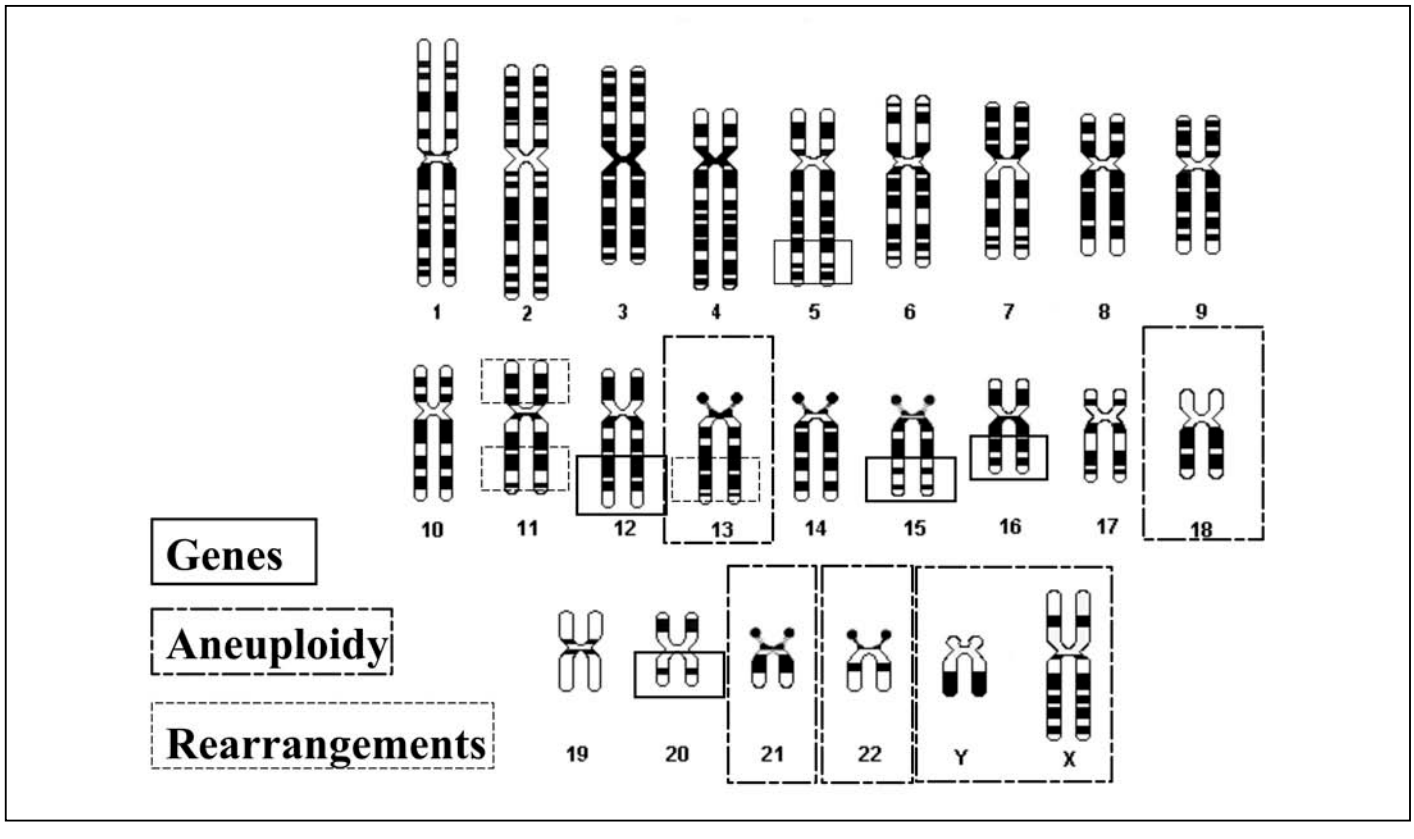


Fig. 3. Genomics of lymphedema-angiodysplasia syndromes displaying mutation of known genes for familial Milroy lymphedema subpopulation (VEGFR-3 at chromosome 5 q34-35), lymphedema-distichiasis (FOXC2 at chromosome 16 q24), and hypotrichosis, lymphedema, telangiectasia (SOX18 at chromosome 20q13) and linkage locations for Aagenaes syndrome (chromosome 15) and Noonan syndrome (chromosome 12). In addition, aneuploidies and rearrangements involving other chromosomes are associated with additional lymphedema-angiodysplasia syndromes. Modified from Witte et al., in *Textbook of Lymphology,* 2003.

TRANSLATION TO CLINICAL LYMPHOLOGY

Regarding treatments: How can or do we approach swelling diseases, scarring diseases, immunodeficiencies, nutritional depletion, and angiogenic syndromes resulting from disruption of the blood lymph loop? Such disorders often have no current treatments, operative or non-operative. Others, such as the edemas, immunodeficiencies, nutritional depletion can be handled but not optimally. Prevention is a distant goal. Right now, the cornerstone remains the "Földi Method", although Michael Földi assures me there is no such thing. It's just tailoring the treatment, by understanding the patient, evaluating through multimodal imaging, and diagnosing and working through each problem, whether a chylous reflux syndrome or Cristobal Papendieck's specialty – the massive, disfiguring lymphangioma-hemangioma syndromes.

The ability to understand the molecular and genetic mechanisms behind lymphangiogenesis/ hemangiogenesis/angiotumor stimulation and inhibition is crucial to halting or reversing these processes when excessive or alternatively enhancing them when deficient. With the aplastic/hypoplastic lymphedemas, the goal is to promote lymphatic growth; and with the uncontrollable lymphangiomas to slow, stop, or reverse the uncontrolled proliferation. These are future directions of therapy, and as I suggested to Michael and Ethel Földi, they will need another suite of rooms in the Földiklinik for the molecular-based and cell-based therapies of the future. Within a few years, there will be an opportunity to evaluate new lymphedema-angiodysplasia treatments, assure that the risks are outweighed by the benefits, and select the best approaches for each patient — "personalized" regimens of designer drugs based on pharmacogenomics and non-invasive image guided delivery systems as well as future tissue engineering and microsurgical approaches (Fig. 5).

Finally, let us return to the place we began, the concept of translational lymphology, moving from molecular lymphology to the clinic, to the practical applications: the screening, classification, evaluation, and treatment of patients as well as the prevention of disease. And the questions (Fig. 6). Genomics begins with finding the genes. But there's a long circuitous pathway from the gene to understanding what's going on and correcting the problems in patients. We need to understand the protein products of the genes, how they're expressed, the sequence of events, the processes, how various systems work together, steps where imaging may help us, system interactions – the lymphatics with the blood vessels, the lymphatic vessels with the rest of the immune system, and a search for disease models either in patients or animals, as we examine the varied phenotypes of the patient, and finally get to restoring health and preventing disease.

This is the dilemma of modern genomics. Regarding the hemoglobinopathies, it has been emphasized how we've learned so much about the genes, the hemoglobins, and the different hemoglobin disorders in man and their genetic basis. But there is

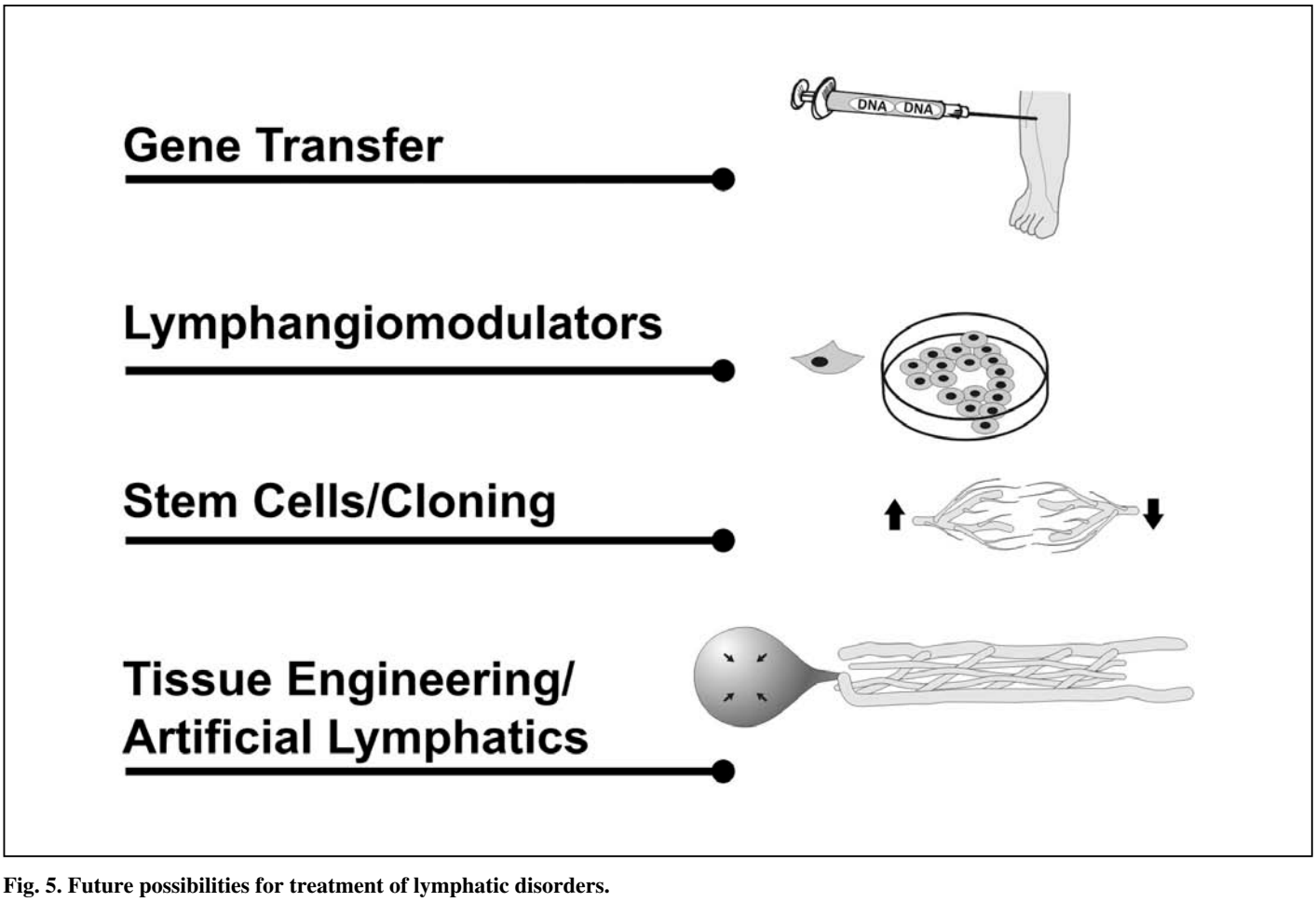


Fig. 5. Future possibilities for treatment of lymphatic disorders.

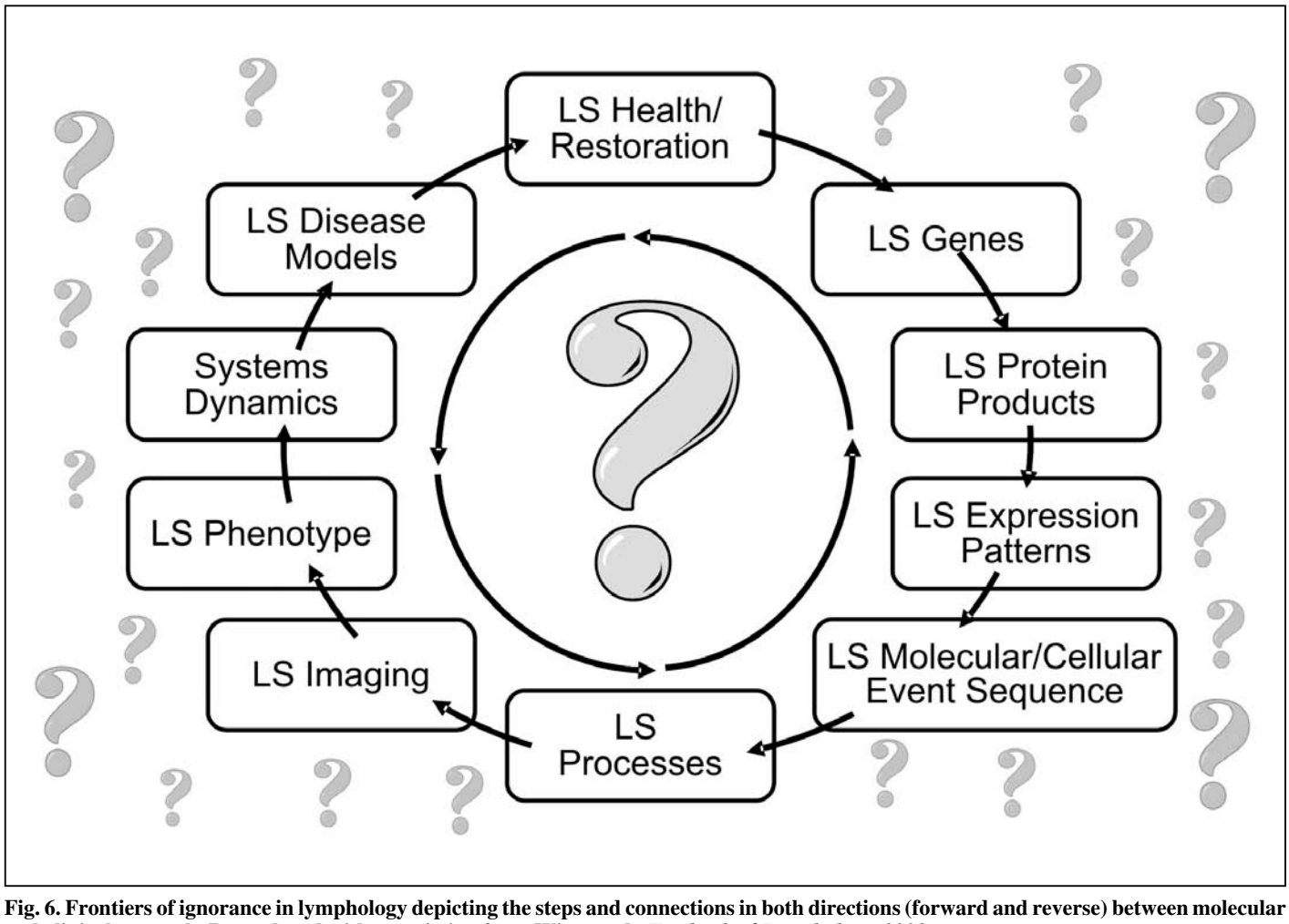


Fig. 6. Frontiers of ignorance in lymphology depicting the steps and connections in both directions (forward and reverse) between molecular and clinical research. Reproduced with permission from Witte et al., *Textbook of Lymphology,* 2003.

nothing we can do with this knowledge to change the course of these diseases and specifically help affected patients. And that is the ultimate paradox and paralyzing dilemma. We should never be boastful of how much we think we understand, for example on the molecular level, until we can translate it into something of value for the patient, who is the ultimate testing ground of the depth and the completeness of our understanding and the point of it all. Furthermore, there is another central question in translational medicine about risks and benefits. Many patients with lymphedema, in fact, most patients in Arizona with leg swelling, are reasonably satisfied by therapy that succeeds in fitting them back into their "jeans." At what point are we ready to offer "gene therapy" as an alternative or adjunct to combined physiotherapy or a variety of operative procedures for "jean therapy"? With one therapeutic candidate, VEGF-C, we (and Institutional Review Boards) would likely be reluctant to approve therapy with this growth factor in a woman with lymphedema after surgery or irradiation for breast cancer, because the very treatment, which has not yet been demonstrated to stimulate proper and significant lymphatic regrowth, might in the long run promote return and spread of the breast cancer. So we are in the quandary, even if VEGF-C were effective in the way and degree it remakes lymphatic vessels (despite the lackluster performance of more potent VEGFs in stimulating blood vessel regrowth in coronary and peripheral vascular disease), we must still consider potential long-term risks, particularly in promoting cancer recurrence. Any member of the vascular-endothelial growth factor family would need to demonstrate beforehand that the postulated benefits outweigh those risks in cancer patients.

TELELYMPHOLOGY

We lymphologists, together with specialists – basic scientists and clinicians – from other disciplines, will continue to explore these unanswered questions, this ignorance – worldwide. Old and new partnerships will bring the latest in genomic and proteomic technology to bear on better delineated clinical problems, and our communication/collaboration system and potential will expand dramatically. Beginning in October 2004 in Genoa, Italy, powerful telemedicine technology arising in the state-of-the-art Arizona Telemedicine Program was tested to link the discipline of lymphology by global telecommunications. Because there are very few societies so international in scope as the ISL, with 44 countries representing the many key basic and clinical specialists in those countries, and treating patients with very visual

abnormalities that can be transmitted on camera, we could actually be sharing our questions and expertise simultaneously all over the world. We could reach remote areas, on Native American reservations, in villages in rural India and on tropical islands. Homes, operating rooms, and laboratories can also be as accessible from the Tucson Telemedicine hub as was Corradino Campisi's and Francesco Boccardo's operating room in Genoa, where the surgeons were viewing a lymphatic-venous shunt procedure live and asking questions in an interactive fashion. So, the use of Telemedicine technology for TeleLymphology, for clinical care, for teleconsultation, for educational purposes, research collaboration and planning clearly is an exciting path of the "next future." The newly expanded Földiklinik will be a premier hub in this new TeleLymphology global alliance within the International Society of Lymphology.

REFERENCES

Evans A.L., Brice G., Sotirova V., Mortimer P., Beninson J., Burnand K., Rosbotham J., Child A., Sarfarazi M. (1999): *Mapping of primary congenital lymphedema to the 5q35.3 region.* Am. J. Hum. Genet., 64: 547-555.

Fang J.M., Dagenais, S.L., Erickson, R.P., Arlt, M.F., Glynn, M.W., Gorski, J.L., Seaver, L.H., and Glover, T.W. (2000): *Mutations in FOXC2 (MFH-1), a forkhead family transcription factor, are responsible for the hereditary lymphedema-distichiasis syndrome.* Am. J. Hum. Genet., 67: 1382-1388.

Ferrell R.E., Levinson K.L., Esman J.H., Kimak M.A., Lawrence E.C., Barmada M.M., Finegold D.N. (1998): *Hereditary lymphedema: Evidence for linkage and genetic heterogeneity.* Hum. Mol. Genet., 7: 2073-2078.

Gale N.W., Thurston G., Hackett S.F., Renard R., Wang Q., McClain J., Martin C., Witte C., Witte M.H., Suri C., Campochiaro P.A., Wiegand S.J., Yancopoulos, G.D. (2002): *Angiopoietin-2 is required for postnatal angiogenesis and lymphatic patterning, and only the latter role is rescued by angiopoietin-1.* Developmental Cell, 3: 411-423.

Iida K., Koseki H., Kakinuma H., Kato N., Mizutani-Koseki Y., Ohuchi H., Yoshioka H., Noji S., Kawamura K., Kataoka Y., Ueno F., Taniguchi M., Yoshida N., Sugiyama T., Miura N. (1997): *Essential roles of the winged helix transcription factor MFH-1 in aortic arch patterning and skeletogenesis.* Development, 124: 4627-4638.

International Society of Lymphology Executive Committee (1995): *The Diagnosis and Treatment of Peripheral Lymphedema.* Lymphology, 28: 113-117.

Irrthum A., Devriend K., Chitayat D., Matthijs G., Glade C., Steijlen P.M., Fryns J.-P., Van Steensel A.M., Vikkula M. (2003): *Mutations in the transcription factor gene SOX18 underlie recessive and dominant forms of hypotrichosis-lymphedema-telangiectasis.* Am. J. Hum. Genet., 72: 1470-1478.

Karkkainen M.J., Ferrell R.E., Lawrence E.C., Kimak M.A., Levinson K.L., McTigue M.A., Alitalo K., Finegold D.N. (2000): *Missense mutations interfere with vascular endothelial growth factor receptor-3 signaling in primary lymphedema.* Nature Genet., 25: 153-159.

Kinmonth, J.B. (Ed.), (1972): *The Lymphatics: Diseases, Lymphography and Surgery.* Edward Arnold, London.

Kriederman B.M., Myloyde T.L., Witte M.H., Dagenais S.L., Witte C.L., Rennels M., Bernas M.J., Lynch M.T., Erickson R.P., Caulder M.S., Miura N., Jackson D., Brooks B.P., Glover T.W. (2003): *Foxc2 haploinsufficient mice are a model for human autosomal dominant lymphedema-distichiasis syndrome.* Human Molecular Genetics, 12: 1179-1185.

Lymphology (1967-2006) Vols. 1-39.

Noon, A., Hunter R.J., Witte M.H., Kriederman B, Bernas M, Rennels M, Percy D, Enerback S, Erickson R.P. (2006): *Comparative lymphatic ocular and metabolic phenotypes of Foxc2 haploinsufficient and aP2-FOXC2 transgenic mice.* Lymphology, 39: 84-94.

Northup K.A., Witte M.H., Witte C.L. (2003): *Syndromic classification of hereditary lymphedema.* Lymphology, 36: 162-189.

Petrova T.V., Karpanen T., Norrmén C., Mellor R., Tamakoshi T., Finegold D., Ferrell R., Kerjaschki D., Mortimer P., Ylä-Herttuala S., Miura N., Alitalo K. (2004): *Defective valves and abnormal mural cell recruitment underlie lymphatic vascular failure in lymphedema distichiasis.* Nature Med, 10: 974-981.

Progress in Lymphology I-XX (1967-2006).

Rusznyák I., Földi M., Szabo G. (1960): *Lymphatics and Lymph Circulation.* Pergamon Press, New York: Oxford, 853 p.

Wilting J., Papoutsi M., Becker J. (2004): *The lymphatic vascular system: Secondary or primary?* Lymphology, 37: 98-106.

Witte C.L., Witte M.H. (2000): *An imaging evaluation of angiodysplasia syndromes.* Lymphology, 33: 158-166.

Witte M.H., Bernas M.J., Northup K.A., Witte C.L. (2003): *Molecular lymphology and genetics of lymphedema-angiodysplasia syndromes.* In: Földi M., Földi E., Kubik S. (Eds). "Textbook of Lymphology". Urban & Fischer Verlag, München, Germany (English text revised by Biotext, LLC, San Francisco), 6th edition, Chapter 16, pp. 471-493.

Witte M.H., Erickson R., Bernas M., Andrade M., Reiser F., Conlon W., Hoyme H.E., Witte C.L. (1998): *Phenotypic and genotypic heterogeneity in familial Milroy lymphedema.* Lymphology, 31: 145-155.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 16 • No. 49 • 2006
INDEXED IN EXCERPTA MEDICA

IMAGING FINDINGS IN PULMONARY LYMPHANGIECTASIA

CARLO BELLINI[1], MD, PHD; FRANCESCO BOCCARDO[2], MD, PHD
CORRADINO CAMPISI[2], MD; EUGENIO BONIOLI[3], MD

[1] Service of Neonatal Pathology, Department of Pediatrics, University of Genoa, Institute G. Gaslini, Genoa, Italy
[2] Department of Surgery, Unit of Lymphatic Surgery, S. Martino Hospital, University of Genoa, Italy
[3] Department of Pediatrics, University of Genoa, Institute G. Gaslini, Genoa, Italy

Correspondence to: Dott. Carlo Bellini
Dipartimento di Pediatria
Università di Genova
Istituto G. Gaslini,
Largo G. Gaslini, 5
16147 Genova
Italy
Tel.: +39 10 5636762
Fax: +39 10 3770675
E-mail: carlobellini@ospedale-gaslini.ge.it

ABSTRACT

We present the imaging findings of 4 newborns and 1 child affected by pulmonary lymphangiectasis (PL). Congenital PL is a rare developmental disorder involving the lung. Pulmonary lymphangiectasis is characterized by pulmonary subpleural, interlobar, perivascular, and peribronchial lymphatic dilatation. Both frequency and etiology are unknown. At birth, PL presents with severe respiratory distress, tachypnea, and cyanosis, with a very high mortality rate at or within a few hours of birth. Bilateral lung reticular appearance, peribronchial cuffing, and bilateral pleural effusions on radiographic chest evaluation are very suggestive of PL. Bilateral septal and peribronchial interstitial thickening are well evidentiated by high-resolution CT. Radiological studies, together with history and clinical data may lead to a diagnosis of PL in most cases. Lymphoscintigraphy, bronchoscopic and pleural effusion evaluation, and if necessary, lung biopsy are useful tools for confirming PL diagnosis.

KEY WORDS: Pulmonary Lymphangiectasia; Imaging findings; Newborn; Child.

INTRODUCTION

Pulmonary lymphangiectasis (PL) is a rare developmental disorder involving the lung, and is characterized by pulmonary subpleural, interlobar, perivascular, and peribronchial lymphatic dilatation. On the basis of an improved characterization of the clinical presentation, and on recent, notable advances in neonatal intensive care, it has been suggested that PL can be divided into two major categories, defined as primary and secondary PL (1, 2).
When presenting as a primary pulmonary developmental defect, PL may be caused by a congenital defect in the primary development of the lung, or may represent a localized expression of more generalized lymphatic involvement.
When it is part of generalized lymphatic dysplasia, PL presents with dilated pulmonary lymphatics truncal lymphangiectasia, which is usually associated with peripheral lymphedema. Hemihypertrophy (which is rare in infants and young children) and diffuse angiomatosis, in which the bone represents the most common site of involvement, can also be observed. Both of these forms may be encompassed by the definition of primary PL. Cardiovascular and lymphatic obstructive forms make up the secondary PL group. Hypoplastic left heart syndrome, pulmonary vein atresia, congenital mitral stenosis, cor triatum, and thoracic duct agenesis are the most likely causes of secondary PL.
Although PL is nearly always fatal during the neonatal period, the recent improvements in mechanical ventilation of severely affected newborns have made survival more frequent. We report our experience in the radiological diagnosis of PL, both in newborns and in older children.

PATIENTS AND RESULTS

Four newborns and 1 child affected by PL were evaluated by imaging between January 2000, and December 2003, at our institute. Newborns who presented at least one sign among the following at birth were examined for the possible presence of pulmonary lymphangiectasia: non-immune hydrops fetalis, hydrothorax, hydropericardium, ascites, lymphedema of the limbs, lymphedema of the genitalia.
Children who presented respiratory difficulties of varying degree with a relapsing course, associated with recurrent cough, wheeze, or inspiratory crackle were examined for the possible presence of pulmonary lymphangiectasia. Diagnostic investigation included

conventional thoracic radiology, HRCT, lymphoscintigraphy, lung biopsy and histology, and pleural effusion evaluation.
Conventional thoracic radiology (5/5 patients), HRCT (4/5 patients), Lymphoscintigraphy (4/5 patients), lung biopsy and histology (2/5 patients) were performed.
Lymphoscintigraphy was performed according to a previously published protocol (3). The diagnosis of chylous effusion was based on the following evaluations: appearance of milk-like fluid, triglyceride level > 110 mL/dL, and lipoprotein analysis for the presence of chylomicrons.
All patients but one are still alive. Age at first examination, prenatal course, clinical course, assistance, and outcome are listed in Table 1. Table 2 summarizes imaging findings.

DISCUSSION

Incidence and etiology of PL are unknown. We reported that congenital PL may be closely related to non-immune hydrops fetalis and to congenital chylothorax (4). It has been suggested that in PL the lymphatic channels of the fetal lung do not undergo the regression process at 20 weeks' gestation, thus large lymphatic vessels that are normal in the 9-16 week gestation maturation developmental process still persist (1). Obstruction of pulmonary lymphatics or veins, or the action of infectious agents have also been evaluated (1).

PL may be associated with, and/or caused by a cardiac lesion. Dilated pulmonary lymphatics develop in utero due to obstructed

Table 1. Summary of the patients' clinical data.

Case	Age when first seen	Prenatal	Assistance	Outcome
1	Birth	Polyhydramnios; Nonimmune hydrops fetalis	Mechanical ventilation; Pleural drainage; Parenteral nutrition	Alive (3 years' follow-up)
2	Birth	Polyhydramnios; Nonimmune hydrops fetalis	Mechanical ventilation and CPAP; Pleural drainage; Parenteral nutrition	Dead
3	Birth	Polyhydramnios, Chylothorax	Mechanical ventilation and CPAP; Pleural drainage; MCT diet; Parenteral nutrition	Alive (2 years' follow-up)
4	Birth	Polyhydramnios; Chylothorax	Mechanical ventilation and CPAP; Pleural drainage; Parenteral nutrition	Alive (28 months' follow-up)
5	20 months	No significant data	Pleural drainage; Parenteral nutrition; MCT diet; Pleurodesis	Alive (6 months' follow-up)

Table 2. Summary of the patients' imaging and diagnostic procedures findings.

Case	Conventional thoracic radiology	HRCT	Lymphoscintigraphy	Biopsy / histology
1	Increased interstitial markings, diffuse thickening of the interstitium, and pleural effusions	Not performed	Dermal back-flow, especially evident at the right lower limb	Not performed (Parents refused)
2	Increased interstitial markings, diffuse thickening of the peribronchovascular interstitium and the septa surrounding the lobules, and bilateral pleural effusion	Diffuse thickening of the peribronchovascular interstitium and the septa surrounding the lobules, associated with bilateral pleural effusion	Rapid drainage of the tracer was especially evident in the lower right limb, suggesting lymphatic hyperplasia. Initial dermal back-flow was evident in the distal portion of the hand and foot, indicating accumulation of the radiocolloid in the dilated channels of the dermis. Back-flow within the thoracic duct was evident	Detected several dilated lymph vessels in the sub-pleural connective tissue, in the interlobular septa, and along bronchovascular bundles (post-mortem)
3	Increased interstitial markings	Diffuse thickening of the peribronchovascular interstitium and the septa surrounding the lobules	Not performed due to clinical condition	Not performed due to clinical condition
4	Peural effusion with mild increased interstitial markings	Large pleural effusion with initial signs related to thickening of the peribronchovascular interstitium	No peripheral lymph vessel abnormalities	Not performed (Parents refused)
5	Increased and diffuse interstitial markings and thickening of the peribronchovascular interstitium and the septa surrounding the lobules with bilateral pleural effusion	Diffuse thickening of the peribronchovascular interstitium and the septa surrounding the lobules with pleural effusion	Mild dermal back-flow was evident in the distal portion of the foot. No back-flow within the thoracic duct was evident	Severely dilated pleural lymph vessels

pulmonary venous flow, or are caused by a cardiac lesion, which has been hypothesized as interfering with normal regression of the lymphatic tissue elements after the 16th week of fetal life.
Chest x-ray evaluation of PL patients usually shows hyperinflation with interstitial markings (Figure 1) (2, 4-10). Longitudinal follow-up pointed to the possible progression of hazy infiltrates, that are usually seen during the neonatal period, to a more perihilar interstitial pattern with varying degrees of lung inflation (1, 2, 8, 9). Generally speaking, it may be affirmed that, like clinical features, radiological findings in PL improve over time.
High Resolution Computed Tomography (HRCT) (2, 4-10) demonstrates diffuse thickening of the interstitium, both of the peribronchovascular interstitium and of the septa surrounding the lobules. In our patients, HRCT showed diffuse interstitial change with thickening of the interlobular septa, which was very often associated with the presence of pleural fluid effusions and atelectasis (Figure 2).
HRCT is the technique of choice for diagnosing PL and, more in general, for diagnosing pediatric interstitial lung disease.
PL may present during the prenatal and /or neonatal period, during childhood, or in adult age.
Any condition that may possibly be related to respiratory distress syndrome of the neonate has to be taken into consideration when making a differential diagnosis (pulmonary aspiration syndrome, interstitial lung disease, pulmonary infection). Furthermore, PL should be taken into consideration in the differential diagnosis of children with chronic respiratory symptoms and with rare pediatric

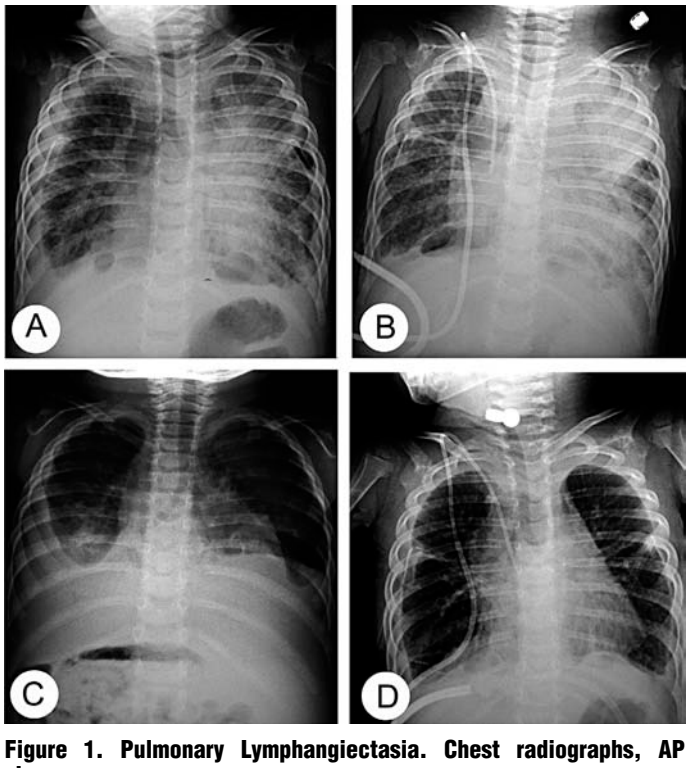


Figure 1. Pulmonary Lymphangiectasia. Chest radiographs, AP views.
Patient 5. In the same patient all the PL radiological findings occurred in the clinical course. A, and B: over time progression of hazy perihilar infiltrates on the left lung. C: important bilateral pleural effusion. D: after pleurodesis, bilateral lung hyperinflation with interstitial and septa thickening are evident, and a mild degree of pleural effusion remains.

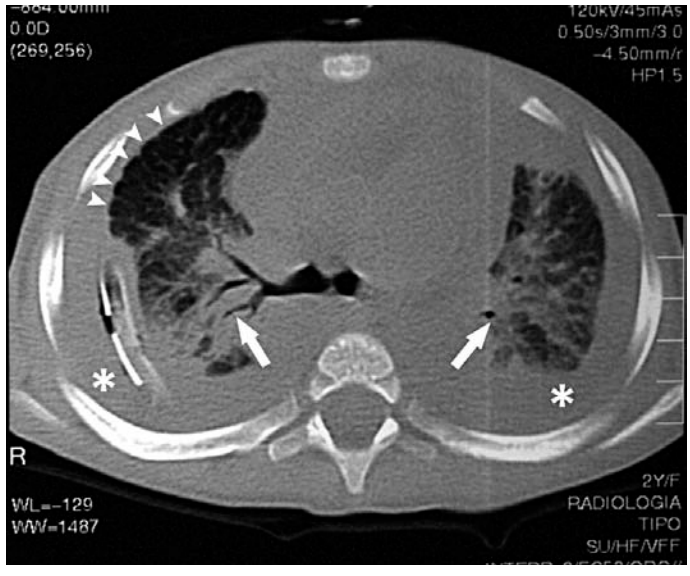


Figure 2. Pulmonary Lymphangiectasia. HRCT.
Patient 2. Diffuse thickening of the peribronchovascular interstitium and the interlobular septa (arrowheads), associated with bilateral pleural effusion (*), and peribronchovascular infiltrates (arrows) with bronchogram.

interstitial lung disease, which is a heterogeneous group of disorders characterized by the presence of inflammation of pulmonary interstitium (11). Several conditions may present with clinical and radiologic signs that are similar to disorders involving lung interstitium. Thus, in differential diagnosis, idiopathic interstitial pneumonitis, follicular bronchiolitis, alveolar proteinosis, lymphocytic interstitial pneumonitis, idiopathic pulmonary hemosiderosis, and lymphangiomatosis must all be taken into consideration.
We suggest paying close attention to all cases of peripheral lymphatic involvement, with or without clinical pulmonary signs, and searching for generalized lymphatic dysplasia.
PL may present at birth as a stillbirth, or with severe respiratory distress, tachypnea, and cyanosis, with a very high mortality rate at or within a few hours of birth (2).
In the post-neonatal period , children with PL present with respiratory difficulties of varying degree, associated with a progressively relapsing course. During both the neonatal and post-neonatal period, PL may be associated with chylothorax, chylopericardium and chylous ascites. During the post-neonatal period it is frequently associated with recurrent cough, wheeze, increased respiratory effort with inspiratory crackle, and in some cases, with congestive heart failure. The disease is characterized by frequent respiratory exacerbations (1, 9).
It can be affirmed that respiratory symptoms may improve in most patients after infancy, and that such symptoms will have notably improved by the age of six. Recent advances in intensive neonatal care have changed the previous, nearly fatal outcome of PL at birth. HRCT scan findings have also shown improvement over time, although the regional pattern of parenchymal inhomogeneity persisted in several studies (10).
Besides imaging studies, other useful diagnostic methods include lung biopsy, lymphoscintigraphy, and bronchoscopic and cavity effusion evaluation, in particular for pleural effusion.

Lung biopsy (9, 10) may be useful for demonstrating the presence of dilated lymphatic spaces in the sub-pleural connective tissue, along thickened interlobar septa, and around bronchovascular axes. Lymphoscintigraphy (2-4, 12) is a minimally invasive technique that provides valuable morpho-functional information regarding the lymphatic system. Lymphoscintigraphy is useful for evaluating lung lymph vessel involvement by showing radiotracer accumulation in the lung and by providing evidence of back-flow within the thoracic duct. It is also useful for evaluating possibly generalized associated lymph vessel dysfunction (Figure 3). Although bronchoscopic evaluation and lung function tests (9, 10) are not specifically indicated in PL, they may be useful for ruling out other pulmonary pathologies and for carrying out bronchial lavage in order to identify and isolate respiratory pathogens. If chylothorax occurs, evaluation of pleural effusion demonstrates triglyceride levels > 1.1 mmol/L and cell counts > 1,000 cells/μL, with a predominance of lymphocytes (approx. 80%) (13, 14). However, this is an unreliable diagnostic test in malnourished patients and in patients not receiving enteral nutrition, including the fetus and occasionally the neonate, since elevated triglyceride levels are frequently not detectable in pleural fluid.
In conclusion, imaging studies are of great importance and value in the PL diagnostic work-up (2, 4-10), even though PL imaging may be similar to pulmonary interstitial emphysema or chronic lung disease imaging findings. The occurrence of bilateral lung reticular appearance, peribronchial cuffing, and bilateral pleural effusions on radiographic chest evaluation are highly suggestive of pulmonary lymphangiectasis. Bilateral septal and peribronchial interstitial thickening are well highlighted by HRCT. Radiological studies, together with history and clinical data may lead to a diagnosis of PL in most cases. Lymphoscintigraphy, bronchoscopic and pleural effusion evaluation and, when necessary, lung biopsy are useful tools for confirming PL diagnosis.

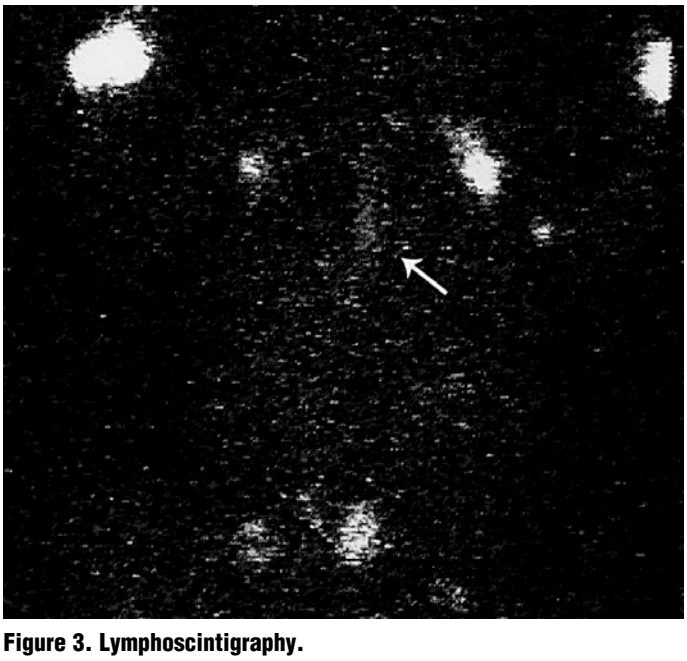


Figure 3. Lymphoscintigraphy.
Patient 1 affected by pulmonary lymphangiectasia and generalized lymphedema. Signs of dermal back-flow were evident at the right lower limb.

REFERENCES

1. Esther CR, Jr. and Barker PM. (2004): *Pulmonary lymphangiectasia: Diagnosis and clinical course.* Pediatr Pulmonol, 38: 308-13.
2. Bellini C, Mazzella M, Campisi C, et al (2004): *Multimodal imaging in the congenital pulmonary lymphangiectasia-congenital chylothorax-hydrops fetalis continuum.* Lymphology, 37: 22-30.
3. Bellini C, Boccardo F, Taddei G, et al (2005): *Diagnostic Protocol for Lymphoscintigraphy in Newborns.* Lymphology, 38: 9-15.
4. Bellini C, Mazzella M, Arioni C, et al (2003): *Hennekam syndrome presenting as nonimmune hydrops fetalis, congenital chylothorax, and congenital pulmonary lymphangiectasia.* Am J Med Genet, 120A: 92-6.
5. Nobre LF, Muller NL, de Souza Junior AS, et al (2004): *Congenital pulmonary lymphangiectasia: CT and pathologic findings.* J Thorac Imaging, 19: 56-9.
6. Chung CJ, Fordham LA, Barker P, et al (1999): *Children with congenital pulmonary lymphangiectasia: after infancy.* AJR Am J Roentgenol, 173: 1583-8.
7. Bouchard S, Di Lorenzo M, Youssef S, et al (2000): *Pulmonary lymphangiectasia revisited.* J Pediatr Surg, 35: 796-800.
8. Huber A, Schranz D, Blaha I, et al (1991): *Congenital pulmonary lymphangiectasia.* Pediatr Pulmonol, 10: 310-3.
9. Barker PM, Esther CR, Jr., Fordham LA, et al (2004): *Primary pulmonary lymphangiectasia in infancy and childhood.* Eur Respir J, 24: 413-9.
10. Copley SJ, Coren M, Nicholson AG, et al (2000): *Diagnostic accuracy of thin-section CT and chest radiography of pediatric interstitial lung disease.* AJR Am J Roentgenol, 174: 549-54.
11. Bokulic RE and Hilman BC. (1994): *Interstitial lung disease in children.* Pediatr Clin North Am, 41: 543-67.
12. Bellini C, Arioni C, Mazzella M, et al (2002): *Lymphoscintigraphic evaluation of congenital lymphedema of the newborn.* Clin Nucl Med, 27: 383-4.
13. van Straaten HL, Gerards LJ, and Krediet TG. (1993): *Chylothorax in the neonatal period.* Eur J Pediatr, 152: 2-5.
14. Staats BA, Ellefson RD, Budahn LL, et al (1980): *The lipoprotein profile of chylous and nonchylous pleural effusions.* Mayo Clin Proc, 55: 700-4.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 16 • No. 49 • 2006
INDEXED IN EXCERPTA MEDICA

DEMONSTRATION OF FLOWAVE'S EFFECTIVENESS THROUGH LYMPHOSCINTIGRAPHY

DOTT. MAURIZIO RICCI, MFT SIMONA PALADINI

U.O. Medicina Riabilitativa, Azienda Ospedaliera Umberto I, Ancona (Italy)

Correspondence to: Dott. Maurizio Ricci
Direttore U.O. Medicina Riabilitativa
Azienda Ospedaliera Umberto I Ancona
Via Conca
60100 Torrette di Ancona (Ancona, Italy)
Tel +39 0715 963178

ABSTRACT

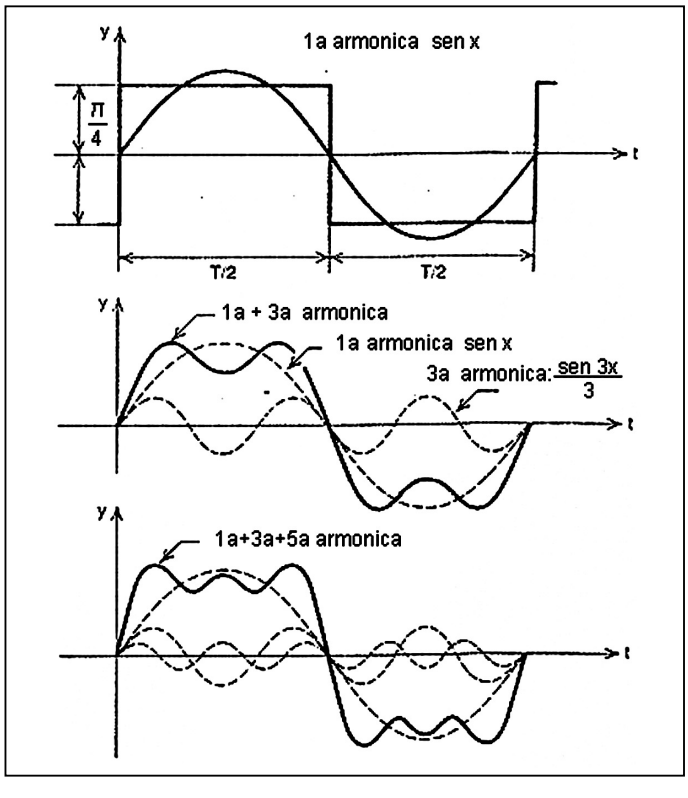
The authors clinically verified the effects and the effectiveness of a new electro-medical instrument named **Flowave**.
This instrument uses mechanical waves to influence interstitial proteins in lymphoedema. The study's protocol (50 patients) used lymphoscintigraphy to verify Flowave's effects.
In conclusion the study demonstrated Flowave stimulates progression lymphatic liquids, activates apical limbs lymph nodes, reduces derma back flow.

KEY WORDS: Sonorous resonance; Lymphoedema; Sound waves.

INTRODUCTION

Flowave is an electro-medical instrument which produces mechanical waves (low frequency sound waves like infrasounds) which are able to interfere in the biological processes of the organism tissue and especially used in treatment of oedema. It was first introduced like an evolution of the MLD, though it is together a "biological" and "mechanical" method. It mechanically stimulates the lymphatic ways and it acts in the treated area through means of a molecular activation.
It works with the activation of the proteins (1-2-8) included those contained in the lymph, according to the physical process of the **sonorous resonance** (3): each sonorous source emits sounds with a characteristic frequency. If it is invested by a sound wave emitted by another source of clearly various frequency, it behaves like a rigid system and it doesn't modify itself. If the frequency is the same or a little different, the source enters in oscillation and starts to emit sounds which reinforce the first sound: this is the resonance. It is famous the example of a violin placed in a room and which enters in sonorous resonance with another violin in the same room, stimulated by a musician.
In the biophysical studies it is considered that the amino-acids behaviour, or the behaviour of a proteic aggregate, is like a system able to oscillate, with its own frequencies. Therefore every time this oscillating system is subordinate to a periodic series of impulses, of equal frequency or nearly, this last one will oscillate (bioresonance) (4-5-6-8-9) with proportional amplitude to the energy by which it was hit. So the structure of amino-acid and/or protein will be pushed towards or into the lymphatic and/or venous system.

Flowave consists of a source which emits a compensated two-phases wave, of amplitude between –12 and +12 Volts. The low energy of this wave does not induce any irreversible modification to the tissues under treatment (7).
The emitted wave consists of a periodic modulated square wave, able (according to Fourier's theorem) to generate an harmonica (wave) with multiple frequencies of the main one. The emission of the main harmonica is never pure but it is always together with other harmonicas, with smaller intensity, whose frequency is generally multiple of the main one. These last ones are said harmonics.
Such harmonics, even if with a minimal amplitude, succeed in stimulating the proteins, and not only: thanks to the inner mechanisms of amplification of the human body, they allow **Flowave** to strengthen several macromolecules with frequency of various resonance (10-11).



A lymphatic oedema is a pathology characterized by an high interstitial proteinous concentration which recalls and keeps water molecules in the interstice and so it favourites fibrosis. For an efficient and long-lasting therapeutic effect it is necessary to remove these substances from the interstice in order to be followed by the water molecules.
Limphoscintigraphical and ultrasonographic studies with high resolution have demonstrated that by using sound waves it is possible to activate interstitial proteinous molecules, allowing their removal. Sound waves also stimulate some intracellular proteins, activating metabolic processes revealed by the expulsion of cellular products (3-5-6-7).
These data caught our attention and induced us to clinically verify the effects and the effectiveness of **Flowave** with lymphoscintigraphy.
So, we set up a protocol with the following **criteria of inclusion**:

- Patients with lymphedema, primary or secondary, mono or bilateral, of the superior or inferior limb;
- Initial stage (at least) 2 months;
- stage of the lymphedema between 2° and 4°;
- they were not receiving to other treatments and the chronic patients observed a therapeutic wash out for at least 6 months.

Criteria of exclusion:

- presence of pacemakers;
- presence of metallic devices in the limb to treat;
- systemic disease;
- pregnancy.

The study **method** was represented by:

- clinical control made by the same operator;
- centimetric measurement (7 points on the superior and inferior limb) made at the beginning and at the end of the cycle treatment with **Flowave**;
- picture of the limbs in the two controls;
- lymphoscintigraphy before and after the treatment always made by the same operator;
- daily therapy only with **Flowave**, all made by the same operator using a standardized program.

The cases report

From 18.12.2002 to 29.11.2004 50 patients have been found with the aforesaid characteristics. Medium age 55 years (22 - 83). Medium insurgence 25 months. 18 of them were treated with radiotherapy and 32 without it.
The aetiology divided them in:

- 10 cases of venous lymphoedema of the inferior limbs;
- 3 primary lymphoedema of inferior limbs;
- 8 secondary lymphoedema of the inferior limbs;
- 28 secondary lymphoedema of the upper limbs post-mastectomy;
- 1 lipolymphoedema of the inferior limbs.

The number of treatments, was variable from 9 to 14; the period between the execution of the first lymphoscintigraphy and the beginning of the therapy never exceeded 2 days while the time between the end of the sitting and the second lymphoscintigraphy was variable from 1 to 7 days.

Verification of results

In all cases the patients have reported a good adaptation to the received treatment. The feeling of gravity and hardening of the limbs has been eliminated in all cases.
Chronic patients stopped any other therapies for about 6 months before starting this treatment..
In the meanwhile the operator positively estimated the methodical execution of the performance.

CLINICAL RESULTS

The clinical control allowed to verify the reduction of the tension of the cutaneous and subcutaneous tissues in 100% of the cases.
The measurement of the centrimetric delta (difference between the two limbs) before and after the therapeutic cycle showed a reduction of 74% (37) divided into:

	N.	N. cases with reduction of the Delta	per cent
Venous Lymphedema	10	10	100
Primary Lymph. of I. L.	3	3	100
Secondary Lymph. of I. L.	8	8	100
Secondary Lymph. of U. L.	28	16	57
Lipolymphedema	1	0	0
Total	**50**	**37**	**74**

Lymphoscintigraphic results

In order to describe the Lymphoscintigraphic exams in this article we explained the results in: PROGRESSION OF THE RADIOISOTOPE, VISUALIZATION OF THE APICAL LYMPHADENS, STAGNATION OF RADIOISOTOPE.
We valued each result between 0 to ++ and compared them before and after treatment.
We obtained:

Progression of the radioisotope

- in 21 of 38 cases (57,3 %) an improvement of the radioisotope progression speed;
- in 16 cases a ++ degree;
- only in 11 of 24 cases (46,6 %) the degree remained 0.

	N.	Improved PTS*	I control			II control		
			0	+	++	0	+	++
VENOUS LYMPHEDEMA	10	**5**/7	4	3	3	1	2	7
PRIMARY LYMPH. OF I. L.	3	**1**/1	1	0	2	0	0	3
SECONDARY LYMPH. OF I. L.	8	**3**/7	6	1	1	3	2	3
SECONDARY LYMPH. OF U. L.	28	**11**/22	13	9	6	7	7	14
LIPOLYMPHEDEMA	1	**1**/1	0	1	0	0	0	1
TOTAL	**50**	**21/**38	**24**	**14**	**12**	**11**	**11**	**28**

(*) The improvement comes from general board. The number under line is number of patients which need improvement.

Visualization of the apical lymph nodes

- In 15 of 39 cases (38,4 %) a better visualization of the apical lymph nodes in the limb was demonstrated;
- In 10 cases we obtained a ++ degree;
- Only in 19 cases the degree remained 0 (12 of them were post-mastectomy).

	N.	Improved PTS*	I control			II control		
			0	+	++	0	+	++
VENOUS LYMPHEDEMA	10	**5**/6	4	2	4	1	1	8
PRIMARY LYMPH. OF I. L.	3	**1**/2	1	1	1	1	0	2
SECONDARY LYMPH. OF I. L.	8	**3**/8	7	1	0	5	2	1
SECONDARY LYMPH. OF U. L.	28	**5**/22	16	6	6	12	7	9
LIPOLYMPHEDEMA	1	**1**/1	0	1	0	0	0	1
TOTAL	**50**	**15/**39	**28**	**11**	**11**	**19**	**10**	**21**

(*) The improvement comes from general board. The number under line is number of improvable patients but secondary lymphoedemas are not valuable by the chirurgical act.

Stagnation of radioisotope (Derma back flow)

- 17 of 34 cases (50%) reduced the radioisotope stagnation;
- 10 cases out of 34 (29,4%) got 0;
- Only 8 out of 22 remained ++ (36,3 %).

	N.	Improved PTS*	I control			II control		
			0	+	++	0	+	++
VENOUS LYMPHEDEMA	10	**5**/7	3	4	3	6	3	1
PRIMARY LYMPH. OF I. L.	3	**2**/2	1	0	2	2	1	0
SECONDARY LYMPH. OF I. L.	8	**0**/2	6	1	1	6	1	1
SECONDARY LYMPH. OF U. L.	28	**10**/23	5	7	16	11	11	6
LIPOLYMPHEDEMA	1	**0**/0	1	0	0	1	0	0
TOTAL	**50**	**17/**34	**16**	**12**	**22**	**26**	**16**	**8**

(*) The improvement comes from general board. The number under line is number of improvable patients.

In conclusion we demonstrated that Flowave can:

- Induce a good progression of the radioisotope in 78 % of the cases;
- Induce an activation of the apical lymph nodes in 62 % of the cases;
- Facilitate a disappearance of ++ derma back flow in 52 % of the cases.

GENERAL BOARD

PRIMARY LYMPH. OF I.L.										
		I CONTROL	II CONTROL		I CONTROL	II CONTROL		I CONTROL	II CONTROL	
		P	P		LN	LN		DBF	DBF	
B.G.	D.	0/+	++	m	0	0	s	++	0/+	m
Q.	D.	++	++	s	+	++	m	++	+	m
B.	A.	++	++	s	++	++	s	0	0	s
VENOUS LYMPHEDEMA										
		I CONTROL	II CONTROL		I CONTROL	II CONTROL		I CONTROL	II CONTROL	
		P	P		LN	LN		DBF	DBF	
A.	M.	0/+	+	m	0	++	m	0	0	s
A.	C.	++	++	s	++	++	s	++	++	s
C.	A.	+	++	m	++	++	s	++	+	m
G.	F.	0/+	++	m	0	++	m	+	0	m
I.	A.	+	++	m	++	++	s	+	0	m
M.	P.	+	+	s	0	+	m	+	+	s
G.	G.	0	0	s	+	++	m	++	+	m
C.	A.	++	++	s	+	++	m	+	0	m
P.	P.	++	++	s	0	0	s	0	0	s
F.	R.	0/+	++	m	++	++	s	0	0	s
SECONDARY LYMPH. OF I.L.										
		I CONTROL	II CONTROL		I CONTROL	II CONTROL		I CONTROL	II CONTROL	
		P	P		LN	LN		DBF	DBF	
C.	M.	0/+	0/+	s	0	+	m	+	+	s
G.	A. M.	0	+	m	0/+	+	m	0	0	s
Q.	O.	0	0	s	0/+	0/+	s	0	0	s
T.	A.	0	++	m	+	++	m	++	++	s
P.	L.	0/+	++	m	0	0	s	0/+	0	s
T.	L.	0/+	0/+	s	0	0	s	0	0	s
G.	S.	+	+	s	0	0	s	0	0	s
S.	M.	++	++	s	0	0	s	0	0	s
SECONDARY LYMPH. OF U.L.										
		I CONTROL	II CONTROL		I CONTROL	II CONTROL		I CONTROL	II CONTROL	
		P	P		LN	LN		DBF	DBF	
A.	M.	0	0	s	0	0	s	++	++	s
B.	R. N.	+	+	s	+	+	s	+	+	s
B.	V.	0	0	s	+	+	s	++	++	s
C.	G.	0	+	m	0	++	m	++	0/+	m
C.	T.	0	0	s	0	0	s	+	+	s
C.	L.	0	++	m	++	++	s	++	0	m
D. C.	V.	++	++	s	+	+	s	++	+	m

SECONDARY LYMPH. OF U.L.										
		I CONTROL	II CONTROL		I CONTROL	II CONTROL		I CONTROL	II CONTROL	
		P	P		LN	LN		DBF	DBF	
L.	P.	0/+	0/+	s	0	0	s	+	+	s
M.	R.	+	++	m	++	++	s	++	0/+	m
M.	M. L.	0	+	m	0	++	m	++	++	s
R.	D.	0/+	++	m	0	0	s	++	+	m
S.	G.	+	++	m	++	++	s	++	++	s
C.	L.	++	++	s	++	++	s	0	0	s
C.	B.	0	0/+	s	0	+	m	++	+	m
L.	G.	++	++	s	++	++	s	0	0	s
B.	A. M.	0	0	s	0	0	s	++	++	s
S.	A.	0	0	s	0	0	s	+	+	s
B.	I.	+	+	s	+	+	s	++	0	m
C.	L.	+	++	m	+	++	m	0	0	s
C.	A.	++	++	s	0	0	s	+	+	s
C.	N.	+	+	s	0	0	s	+	+	s
R.	A. B.	+	++	m	0	0	s	++	0	m
R.	G.	+	++	m	0	+	m	0	0	s
C.	E.	0	++	m	0	0	s	++	+	m
B.	E.	++	++	s	+	+	s	+	+	s
A.	F.	+	+	s	0	0	s	++	++	s
T.	M. C.	0	+	m	0	0	s	0	0	s
T.	G.	++	++	s	++	++	s	++	0/+	m
LIPOLYMPHEDEMA										
		I CONTROL	II CONTROL		I CONTROL	II CONTROL		I CONTROL	II CONTROL	
		P	P		LN	LN		DBF	DBF	
P.	A.	+	++	m	+	++	m	0/+	0/+	s

Conclusive considerations

- These results are acceptable in order to estimate the executed protocol and **Flowave** efficacy;
- the methodical demonstrated to be able to give positive answers to the clinical plan. This also agrees with the standard parameters considerated by all the operators and guidelines;
- the application of **Flowave** was very appreciated either by the operators or by the patients;
- in chronic patients (who had received a decongestive therapy) was demonstrated a tendency to maintain the obtained results longer.

REFERENCES

1. Bistolfi F. (1989): *Radiazioni Non Ionizzanti, Ordine, Disordine e Biostrutture.* Ed. Minerva Medica, Torino.
2. Breithaupt H. (1989): *Biological rhytms and communication.* In: "Electromagnetic Bio-Information" (F.A. Popp et al., eds.) Urban & Schwarzenberg, Munchen: 18-44.
3. Burr H.S. (1935): *The Electrodynamic theory of life.* Quarterly Review of Biology, 10: 322-333 – Orida N. (1988): *Directional protrusive pseudopodial activity and motility in macrophages induced by extracellular electric fields.* Cell. Motil. 2(3): 243-255.
4. Del Giudice E., Doglia S., Milani M. and Vitiello G. (1988): *Structures, correlations end electromagnetic interactions in living matter: Theory and applications.* In: "Biological Coherence and Response to External Stimuli" (H. Frohlich, ed.) Springer-Verlag, Berlin-Heidelberg: 49-64.
5. Ho M.W., Popp F.A. and Warnke U. (1994): *Bioelectrodynamics and Biocommunication.* World Scientific, Singapore.
6. Ho M.W. (1996): *Bioenergetics and the coherence of the organism.* In: "Hoomopathie-Bioresonanztherapie" (P.C. Endler and J. Schulte, eds.), Verlag Maudrich, Wien: 17-34.
7. Kaada B. (1982): *Treatment of peripheral circulatory disorders and chronic ulceration by transcutaneous nerve stimulation.* Tidsskr nor laege 102(30): 1563-1570.
8. Lubert Stryer: *Biochimica.* Ed. Zanichelli.
9. Popp F.A., Warnke U., Konig H.I. and Peschla W. (eds.) (1989): *Electromagnetic Bio-Information.* Urban and Schwarzenberg, Munchen.
10. Reed B.V. (1988): *Effects of high voltage pulsed electrical stimulation on microvascular permeability to plasma proteins.* In: "Phisical Therapy", 68: 491-495.
11. Tzong T.Y. and Gross C.J. (1994): *The language of cells-Molecular processing of electric signals by cell membrane.* In: "Bioelectrodynamics and Biocommunication" (M.W. Ho et al., eds.) World Scientific, Singapore: 131-158.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 16 • No. 49 • 2006
INDEXED IN EXCERPTA MEDICA

EARLY OR LATE DIAGNOSIS OF LYMPHEDEMA IN OUR LYMPHEDEMA UNIT

ISABEL FORNER-CORDERO, RAQUEL NAVARRO-MONSOLIU*, JOSE MUÑOZ-LANGA**, PILAR REL-MONZÓ**

* Lymphedema Unit. Rehabilitation Department. Hospital Universitario La Fe
** Medical Oncology Unit. Hospital Universitario Dr. Peset. Valencia. Spain

Correspondence to: Isabel Forner-Cordero
C/ Andrés Mancebo 36, 12
46023 Valencia
Spain
Phone: 00-34-649179852
E-mail: iforner@saludalia.com

ABSTRACT

Aim: to describe the characteristics of the patients admitted to the Lymphedema Unit of Hospital Universitario La Fe of Valencia (Spain).

Material and method: A descriptive study of patients seen in our Unit from September 2005 to March 2006 was performed. The epidemiological and clinical features were recorded.

Results: A total of 475 lymphedema patients were included, 310 cases (65.3%) with Upper Limb Lymphedema (95.8% of them were secondary to breast cancer treatment), and 166 cases (34.9%) with Lower Limb Lymphedema (70.6% of them were primary). The degree of the disease was more severe in Lower Limb Lymphedema than in Upper Limb ($p<0.0001$). Dermal complications were present in 27.6% of the patients with Upper Limb Lymphedema and in 55.5% of the patients with Lower Limb Lymphedema ($p<0.0001$). The most common cause for lymphedema in our country is postmastectomy lymphedema, and the patients are referred to us a mean of 2.4 years after the beginning of the symptoms, and in early stages. For the Lower Limb Lymphedema, the referral to our Unit was a mean of 13.4 years after the beginning of the symptoms, and usually in advanced and complicated stages.

Conclusions: These data suggest that Upper Limb Lymphedema is a well-known and expected sequel of breast cancer treatment, and in the case of the Lower Limb Lymphedema it is ignored and usually not properly diagnosed by medical community. Some efforts have to be made to improve medical knowledge in this field, in our country.

KEY WORDS: Lymphedema, epidemiology, staging, complications.

INTRODUCTION

Lymphoedema is a chronic and progressive condition that results from any reduction in the capacity of the lymphatic system to drain fluid from the interstitium and return it to the blood circulation.[1] Lymphedema can be Primary, when produced by congenital abnormalities of the lymphatic system, or Secondary to Cancer and its treatment, traumatism, Filarial infection or Chronic Venous Disease as the main causes. Lymphedema following treatment for breast cancer is the most frequent type of lymphedema in our country, being its incidence from 6 to 30%[2] depending from the sources.

Lymphoedema leads to aesthetic problems, disfunction due to pain, weight and limited mobility, and psychological troubles. Most frequent complications[1] are: infections as erysipela-lymphangitis, fibrosis, elephantiasis, lymph fistulae and lymphatic ulcers.

In the Hospital La Fe of Valencia, the Lymphedema Unit belongs to the Rehabilitation Department and its task is to study and treat the patients affected of lymphedema. This Unit receives patients from Valencia city and the Region of Valencia and often patients from other Spanish regions.

Since the start of the Breast Cancer Unit, that is a multidisciplinary working group for the best management of the breast cancer patients, the referral of the patients from oncologist specialist, radiotherapists and surgeons has improved.

The aim of the study was to describe the characteristics of the patients admitted to the Lymphedema Unit of Hospital La Fe of Valencia (Spain) and the differences between Upper and lower limb Lymphedema patients.

MATERIAL AND METHODS

A descriptive study of patients seen in our Unit from September 2005 to March 2006 was performed. The epidemiological and clinical features were recorded.

The diagnosis of lymphedema was done by the natural history, the clinical exam and the complementary exams as blood biochemistry parameters, echo-dupplex scan, Computed Tomography scan and lymphoscintigraphy findings when possible.[3,4,5,6]

The variables examined were:

a) demographic and epidemiological characteristics: age, sex, weigth and body mass index (BMI);
b) clinical characteristics of lymphedema: upper or lower limb, etiology, onset of lymphedema, limb volume, degree of severity, presence of fibrosis, dominant or non dominant limb, chronicity (in years), history of hypertension, reduced joint mobility, the presence of peripheral nervous impairment, associated syndromes; in secondary lymphedema: surgical intervention, lymphadenectomy, radiotherapy, hormonal therapy; and in primary lymphedema: lymphoscintigraphic data.

The mean of Body Mass Index [7] (BMI) (weight in kg divided by height in m^2) was calculated for all the patients. A normal BMI is defined as 18.5 to 24.9 kg/m^2, overweight is a BMI of 25.0 to 29.9 kg/m^2, obesity is a BMI of 30.0 to 39.9 kg/m^2, and morbid obesity is a BMI > 40 kg/m^2.

The patients were classified following the International Society of Lymphology (ISL) lymphedema staging: [8,9]

– ISL stage 0: A subclinical state where swelling is not evident despite impaired lymph transport;
– ISL stage I: This represents early onset of the condition where there is accumulation of tissue fluid that subsides with limb elevation. The oedema may be pitting at this stage;
– ISL stage II: Limb elevation alone rarely reduces swelling and pitting is manifest;
– ISL late stage II: There may or may not be pitting as tissue fibrosis is more evident;
– ISL stage III: The tissue is hard (fibrotic) and pitting is absent. Skin changes such as thickening, hyperpigmentation, increased skin folds, fat deposits and warty overgrowths develop.

RESULTS

A total of **475 lymphedema** patients were included, 96.4% female and 3.6% male, with a median age of 57.4 years (range: 0-89) (Table 1).

The lymphedema **affected the Upper Limb** in 310 cases (65.3%); the 95.8% of them were secondary to breast cancer treatment (Figure 1). The dominant side was affected in 51.8% of the patients.

The **Lower Limb was affected** in 166 cases (34.9%), 70.6% of them were primary and 29.4% secondary (Figure 1). The genitals were involved in 13 cases (7.8%).

Epidemiological and clinical characteristics:

The differences between Upper and Lower Limb Lymphedema are described in Table 1.

Bilateral affectation was more frequent in Lower Limb Lymphedema (58.4%) than in Upper Limb (2.2%). Patients with Upper Limb Lymphedema were older (mean of age: 57.9, CI 95%: 56.6-59.3) than patients with Lower Limb disease (mean: 50.9, CI 95%: 48.0-53.7) ($F=29.91$; $p<0.0001$). The mean of Body Mass Index [7] (BMI) was higher in Lower Limb Lymphedema patients (Table 1).

The frequency of patients with overweight was greater in Upper Limb Lymphedema, but the frequency of patients with morbid obesity was greater in Lower Limb Lymphedema patients (Table 1).

The mean of duration of lymphedema (chronicity) from the onset of the symptoms until the first visit in our Unit was 2.4 years (CI 95%: 1.8-3.1) for Upper Limb Lymphedema, and 13.4 years

Table 1. Epidemiological and clinical characteristics.

	Total	Upper Limb	Lower Limb	p value
Number of patients	475	310 (65.3%)	166 (34.9%)*	
Age (median; range)	57.4 (0-89)	58.6 (0.7-85.3)	53.7 (0-88.9)	p <0.001
Sex:				
Males	17 (3.6%)	1 (0.3%)	15 (9.0%)	
Females	458 (96.4%)	309 (99.7%)	151 (91.0%)	
Cause:				
Primary	128 (26.9%)	13 (4.2%)	115 (70.6%)	p <0.001
Secondary	345 (72.6%)	297 (95.8%)	48 (29.4%)	
BMI (mean; CI 95%)	29.7 (29.1-30.4)	29.0 (28.4-29.6)	31.1 (29.7-32.6)	p <0.001
Nutricional status:				
Normal	103 (24.9%)	68 (24.3%)	35 (26.1%)	
Overweight	137 (33.1%)	103 (36.8%)	34 (25.4%)	p <0.001
Obesity	137 (33.1%)	98 (35%)	39 (29.1%)	
Morbid obesity	35 (8.5%)	10 (3.6%)	25 (18.7%)	
Affected limb:				
Right	181 (38.1%)	149 (48.1%)	32 (19.3%)	
Left	191 (40.2%)	154 (49.7%)	37 (22.3%)	
Bilateral	104 (21.8%)	7 (2.2%)	97 (58.4%)	
Chronicity (years) (mean; CI 95%)	6.2 (5.2- 7.2)	2.4 (1.8-3.1)	13.4 (11.1- 15.6)	p <0.001

(*) In one case lymphedema was present in upper and lower limbs.

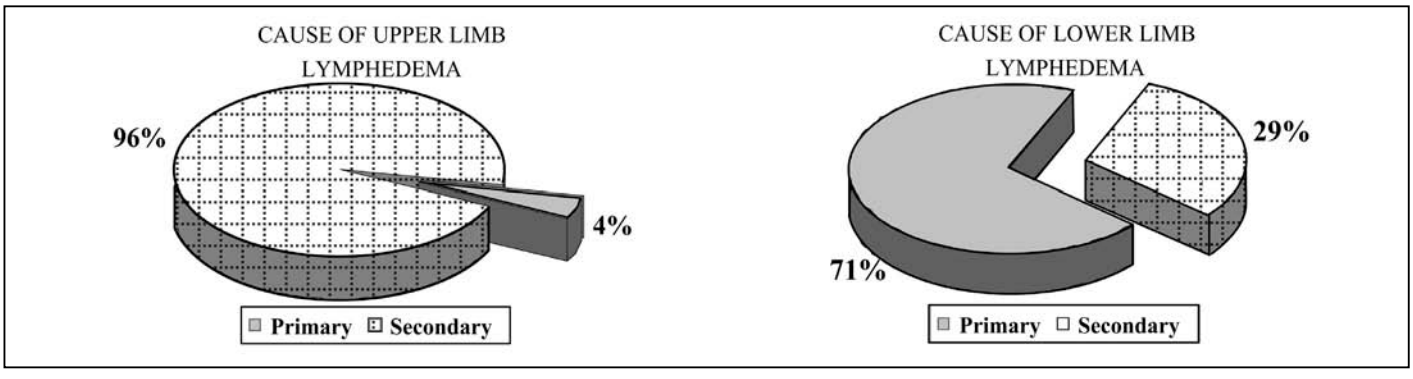


Figure 1 - Etiology of upper and lower limb lymphedema.

(CI 95%: 11.1-15.6) for Lower Limb Lymphedema (range: 0-55) ($F=136.9$; $p<0.0001$) (Table 1). Note that other studies measure the delay in seeking treatment in weeks while we do it in years from the onset.[10]

Etiologic diagnosis:

In Upper Limb the most frequent etiology was postmastectomy lymphedema that was affecting the 96% of them. Only 4% were primary (Table 2).

In Lower Limb, 62% of the patients suffered from Lymphedema, 22.9% of them had Lipedema or Lipolymphedema, 13.8% Phlebo-lymphostatic edema and there were 2 cases with Cystic Lymphangioma (Table 2).

We found some **syndromes associated** with the clinical case (Table 3): Familiar lymphedema affecting one or more members in 16.9%; Familiar lipodystrophy in 14.5%; Rheumatologic diseases as Rheumatoid Arthritis or Systemic Lupus Erythematosus in 7.5%; and other rare diseases: Klippel-Trenaunay syndrome (3 cases); Turner's syndrome (3 cases); Malignant lymphedema (2 cases); Goltz syndrome (1 case); Prader-Willi syndrome (1 case) and Ehler-Danlos syndrome (1 case).

International Society of Lymphology (ISL) lymphedema staging:

The degree of the disease was more severe in Lower Limb than in Upper Limb Lymphedema ($\chi^2 = 59.927$; $p < 0.0001$) (Table 4).

Dermal complications:

They were present in 27.6% of the patients suffering Upper Limb Lymphedema and in 55.5% of the Lower Limb Lymphedema ($\chi^2 = 30.721$; $p < 0.0001$) (Table 5).
One third of the patients had fibrosis in their limbs with a local and limited affectation or in an extended area. The fibrosis was wider and more severe in Lower Limb Lymphedema patients (Table 5). All the types of dermal complications: Lymphedema-related acute dermatitis, Hyperkeratosis, Lymph cysts and Ulcers were more frequent in Lower Limb Lymphedema patients (Table 5).

Table 2. Clinical Diagnosis.

	%	Figures*
Upper Limb:		
Postmastectomy	96	2, 3, 4, 5
Primary	4	
Lower Limb:		
Lymphedema	62	6, 7, 8, 9
Lipedema or	22.9	10
Lipolymphedema	13.8	11,12,13
Phlebo-lymphostatic edema		
Cystic Lymphangioma (2 cases)	1.3	

(*) *See figures related to clinical diagnosis.*

Table 3. Associated syndromes.

	%	Figures*
Familiar lymphedema	16.9	6
Familiar lipodystrophy	14.5	12
Rheumatologic diseases	7.5	
Other rare diseases:	6.6	
Klippel-Trenaunay syndrome (3 cases)		
Turner's syndrome (3 cases)		
Malignant lymphedema (2 cases)		
Goltz syndrome (1 case)		
Prader-Willi syndrome (1 case)		
Ehler-Danlos syndrome (1 case)		

(*) *See figures related to associated syndromes.*

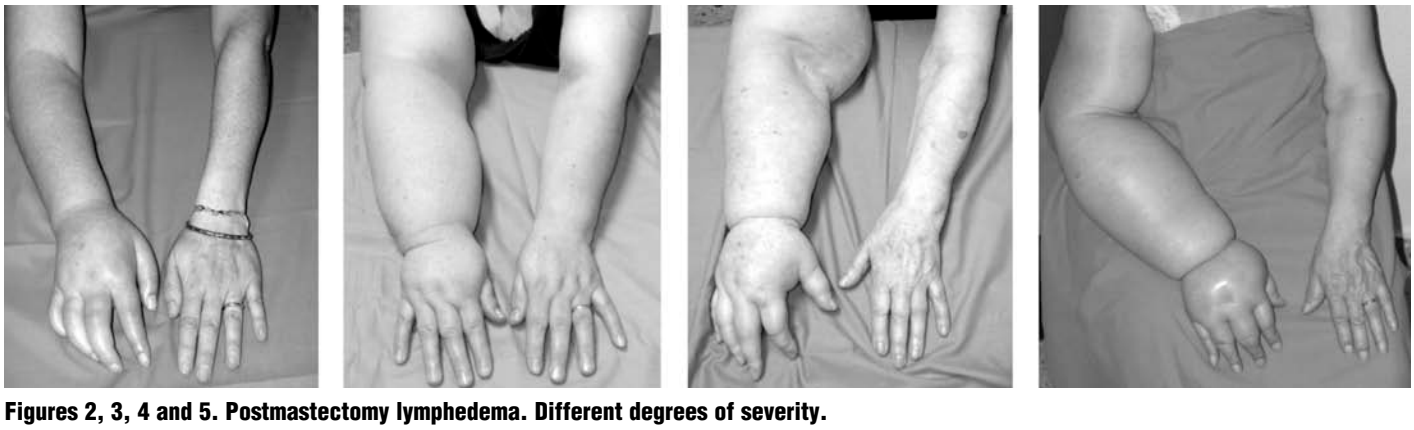
Table 4. International Society of Lymphology lymphedema stages.

Stage	Total	Upper Limb	Lower Limb	Figures*
0	43 (9.1%)	12.9%	1.8 %	
I	173 (36.6%)	40.6%	28.0%	
II	180 (38.1%)	38.7%	37.8%	2, 3, 6, 12
III elephantiasis	77 (16.3%)	7.7%	31.1%	4,5,7,8,9, 10, 11, 13

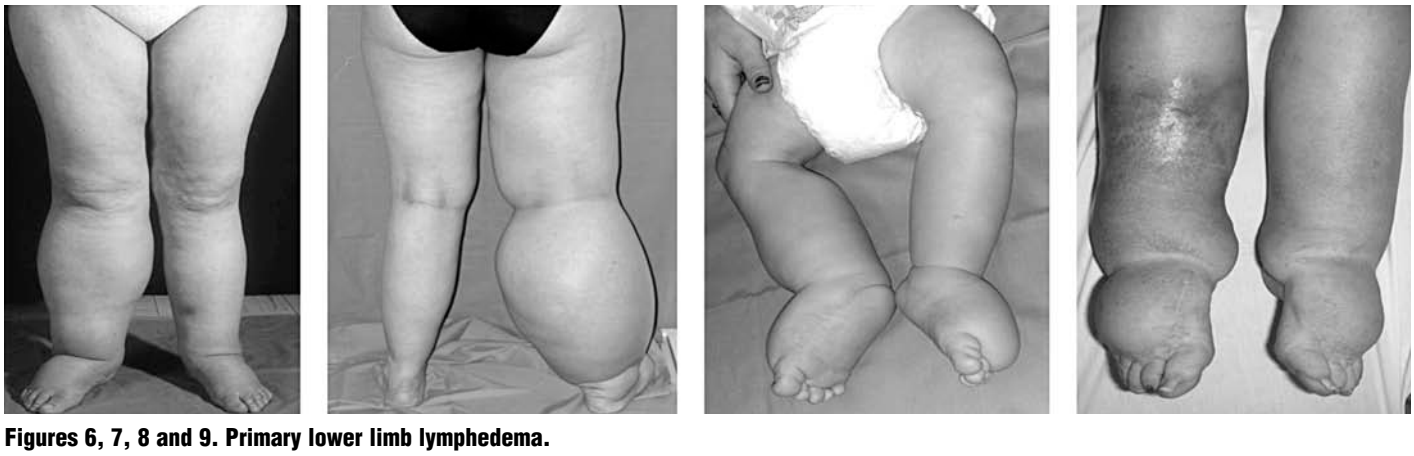
(*) *See figures related to the stages.*

Table 5. Dermal complications of lymphedema.

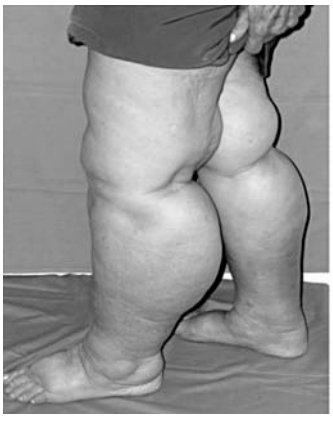
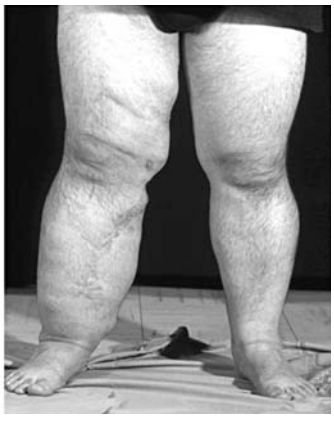
	Total	Upper Limb	Lower Limb	p value
Number of patients	475	310 (65.3%)	166 (34.9%)*	
Dermal complications	174 (36.6%)	83 (27.6%)	91 (55.5%)	p< 0.001
Fibrosis: *Local* *Extended*	 100 (21.1%) 52 (10.9%)	 61 (19.7%) 15 (4.8%)	 36 (22.4%) 37 (23.0%)	 p< 0.001
Lymphedema-related acute dermatitis	105 (22.1%)	35 (11.6%)	71 (43.3%)	p< 0.001
Hyperkeratosis	28 (5.8%)	2 (0.7%)	26 (15.9%)	p< 0.001
Lymph cysts	53 (11.1%)	11 (3.6%)	42 (25.6%)	p< 0.001
Ulcers	28 (5.8%)	1 (0.3%)	27 (16.5%)	p< 0.001
(*) *In one case lymphedema was present in upper and lower limbs.*				



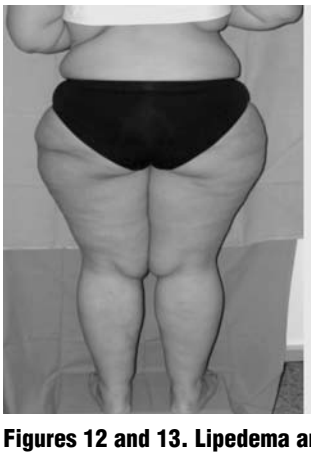
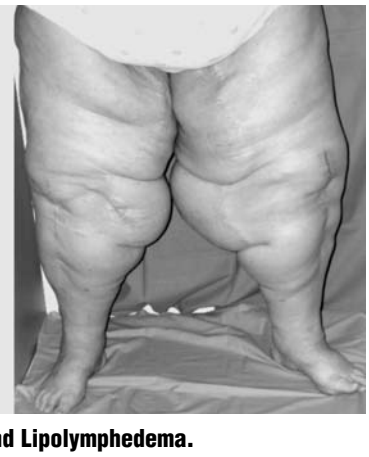
Figures 2, 3, 4 and 5. Postmastectomy lymphedema. Different degrees of severity.



Figures 6, 7, 8 and 9. Primary lower limb lymphedema.

Figures 10 and 11. Secondary lower limb lymphedema.

Figures 12 and 13. Lipedema and Lipolymphedema.

CONCLUSIONS

The most common cause for lymphedema in our country is postmastectomy lymphedema, although the number of Lower Limb Lymphedema admitted in our Unit is increasing nowadays. The patients with Upper Limb Lymphedema are referred to us a mean of 2.4 years after the beginning of the symptoms, and in early stages. This is more evident since our participation in the Breast Cancer Unit that makes other specialists be more aware to detect lymphedema, that is a well-known and expected sequel of breast cancer treatment and to refer them to our Lymphedema Unit.

For the Lower Limb Lymphedema, the referral to our Unit was a mean of 13.4 years after the beginning of the symptoms, and usually in advanced and complicated stages. On the contrary to the Upper Limb, Lower Limb Lymphedema is ignored and usually not properly diagnosed by medical community. Some efforts have to be made to improve medical knowledge in this field, in our country.

REFERENCES

1. Földi E., Földi M.: *Lymphostatic diseases.* In: Földi M. "Texbook of lymphology for physicians and lymphedema therapists". Urban & Fisher. Munchen, 2003. p. 231-319.
2. Kissin M.W., della Rovere G.Q., Easton D. et al.: *Risk of lymphoedema following treatment of breast cancer.* Br J Surgery, 1986; 73: 580-4.
3. Szuba A., Shin W.S., Strauss H.W., Rockson S.: *The third circulation: radionuclide lymphoscintigraphy in the evaluation of lymphedema.* J Nucl Med., 2003; 44: 43-57.
4. Mégret G.: *L'insuffisance lymphatique des membres supérieurs en questions. Angiologie.* Actualités médicales internationales, 1996.
5. Brautigam P., Foldi E., Schaiper I., Krause T., Vanscheidt W., Moser E.: *Analysis of lymphatic drainage in various forms of leg edema using two compartment lymphoscintigraphy.* Lymphology, 1998; 31(2): 43-55.
6. Breu F.X., Marshall M.: *Neue ergebnisse der duplexsonographischen diagnostik des lip- und lymphodems: kompressionssonographie mit einer neven 13-mhz-linearsonde. [New results of the duplex-sonographic diagnosis of the lip- and lymphedema: Compression sonography with a new 13 MHz linear array].* Phlebologie, 2000; 29(5): 124-8.
7. Denke M., Wilson J.D.: *Assessment of nutritional status.* In: Fauci A.S., Braunwald E., Isselbacher K.J., et al. (editors) "Harrinson's principles of internal medicine". 14th ed. New York: McGraw-Hill; 1998. p. 448-52.
8. International Society of Lymphology. *The diagnosis and treatment of peripheral lymphedema. Consensus document of the International Society of Lymphology.* Lymphology, 2003; 36(2): 84-91.
9. *Lymphoedema Framework. Best Practice for the Management of Lymphoedema. International consensus.* London: MEP Ltd, 2006.
10. Iker E., Glass E.: *Responses to tretament in primary and secondary lymphedema: effects of body mass index and delays in recognition.* The European Journal of Lymphology and related problems, 2006; 16 (47):10-3.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 16 • No. 49 • 2006
INDEXED IN EXCERPTA MEDICA

ROLE OF LYMPHOSCINTIGRAPHY IN THE INDICATIONS TO MICROSURGICAL TREATMENT OF PERIPHERAL LYMPHEDEMAS

FRANCESCO BOCCARDO, CARLO BELLINI, CORRADO CAMPISI, COSTANTINO ERETTA, D. PERTILE, EMANUELA BENATTI, MIRKO CAMPISI, GIUSEPPINA TALAMO, ALBERTO MACCIÒ, CORRADINO CAMPISI*

Department of Surgery – Section of Lymphatic Surgery and Microsurgery
S. Martino Hospital, University of Genoa, Italy
* Center of Neonatal Pathology – G. Gaslini Institute, Genoa, Italy

In collaboration with the Unit of Nuclear Medicine of the University of Genoa, Italy

ABSTRACT

The value of lymphoscintigraphy is very high in those cases of early stage peripheral lymphedemas, because it allows to study both superficial and deep lymphatic circulation in a very precise and not only functional but also morphological way, giving important data to the surgeon to establish the correct therapeutic strategy.
Lymphoscintigraphy, in fact, represents the common diagnostic examination which is usually performed by lymphedema patients addressed to an operation of lymphatic microsurgery, together with the study of venous circulation by means of duplex scan.
In the most advanced cases of lymphedema, lymphoscintigraphy proved not to be so reliable, as the tracer remains at the site of injection without any sign of progression. This outcome might lead to a misunderstanding of the etiology and physiopathological mechanism of the pathology, which can be wrongly interpreted as congenitally based on a condition of lymphatic and lymphnodal aplasia (so called Nonne-Milroy disease).
Thus, it is useful to underline how there might exist some cases of "dumb lymphoscintigraphy" notwithstanding the presence of proper lymphatic-lymphnodal structures.

KEY WORDS: Lymphoscintigraphy, lymphedema, microsurgery.

INTRODUCTION

Lymphoscintigraphy represents the common diagnostic examination which is usually performed by lymphedema patients addressed to an operation of lymphatic microsurgery, together with the study of venous circulation by means of duplex scan.
It is indispensable to study the conditions of vein circulation in order to decide which microsurgical technique is to use in the single case, derivative lymphatic-venous shunt or reconstructive lymphatic-venous-lymphatic plasty.

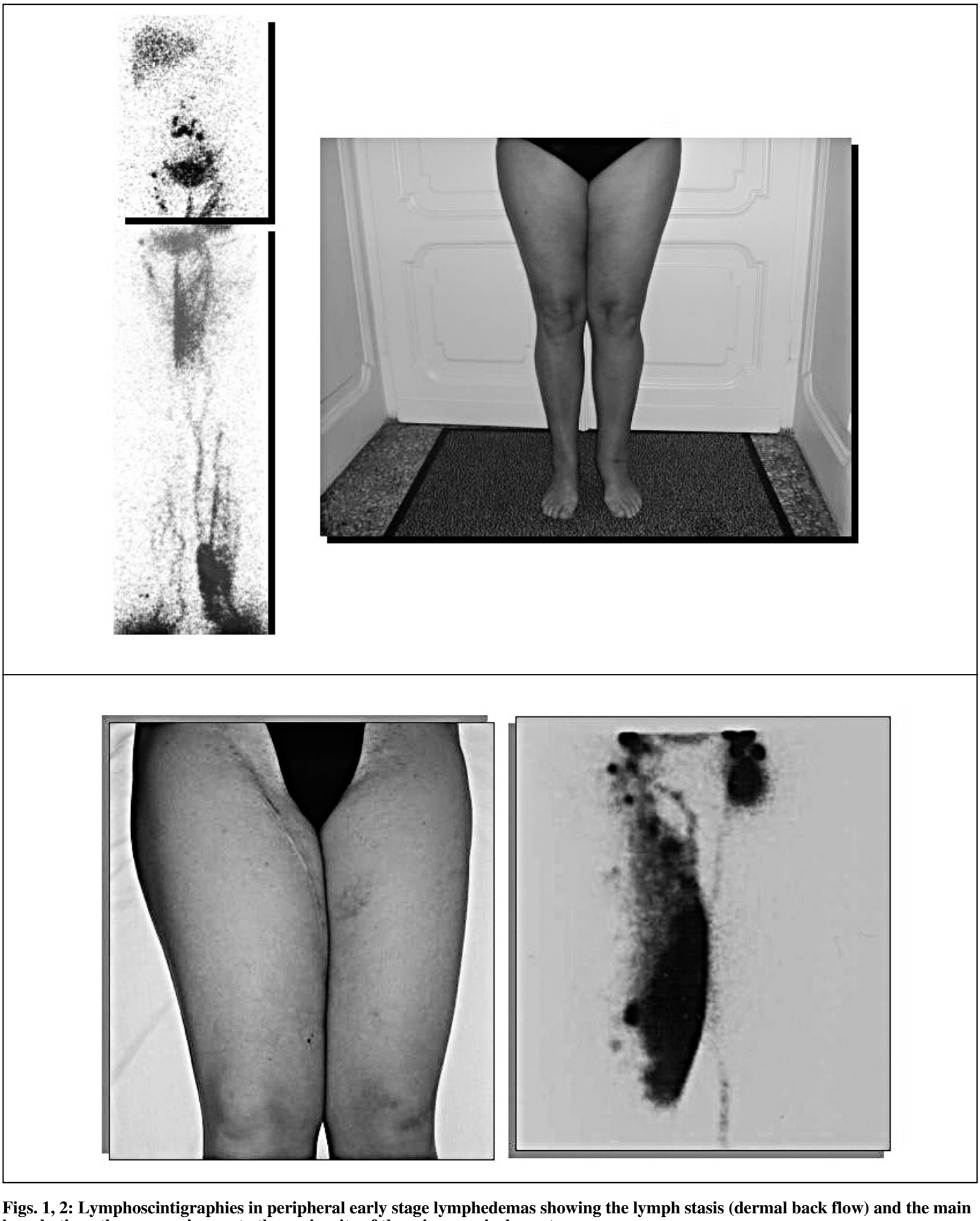
CLINICAL EXPERIENCE

As concerns lymphoscintigraphy,[1-6] the value of this investigation is very high in those cases of early stage peripheral lymphedemas, because it allows to study both superficial and deep lymphatic circulation in a very precise and not only functional but also morphological way, giving important data to the surgeon to establish the correct therapeutic strategy (Figs. 1, 2).

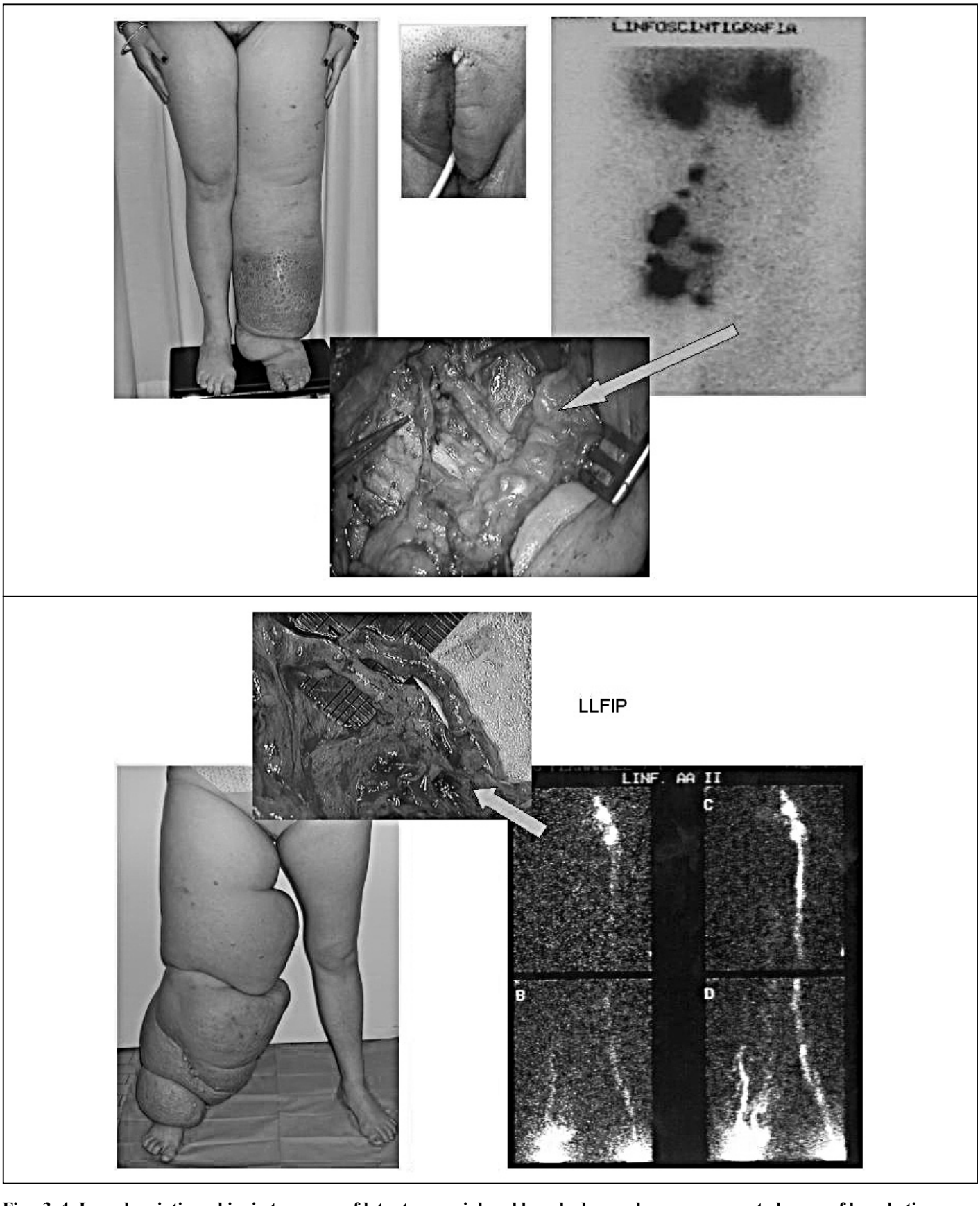
But, in the most advanced cases of lymphedema, lymphoscintigraphy[7-12] proved not to be so reliable, as the tracer remains at the site of injection without any sign of progression. This outcome might lead to a misunderstanding of the etiology and physiopathological mechanism of the pathology, which can be wrongly interpreted as congenitally based on a condition of lymphatic and lymphnodal aplasia (so called Nonne-Milroy disease). On the contrary, it is only a problem of extremely difficult absorption of the tracer by the initial lymphatics, due above all to high interstitial pressure and tissular fibrosis (Figs. 3, 4).

There are, however, sometimes even early stages in which the tracer does not progress from the site of injection, but in these cases we might think of a sort of inhibitory nervous signal from the proximal lymph nodes as it is for the kidney in case of ureteral stone obstruction: we called the phenomenon lymphatic-lymphnodal functional inhibiting phenomenon (LLFIP) (Fig. 5).

The assessment of these cases was wrong by lymphoscintigraphy because when we operated on these patients we could find both lymphatic and lymph nodal structures, not pointed out by lymphoscintigraphy. The problem was of a lymph nodal fibrosclerosis but with good afferent lymphatic collectors that were anastomosed to a near vein (Fig. 6).



Figs. 1, 2: Lymphoscintigraphies in peripheral early stage lymphedemas showing the lymph stasis (dermal back flow) and the main lymphatic pathways coming up to the groin, site of the microsurgical anastomoses.



Figs. 3, 4: Lymphoscintigraphies in two cases of late stage peripheral lymphedemas show an apparent absence of lymphatic-lymphnodal structures that were in fact found at the time of surgery (dumb lymphoscintigraphies).

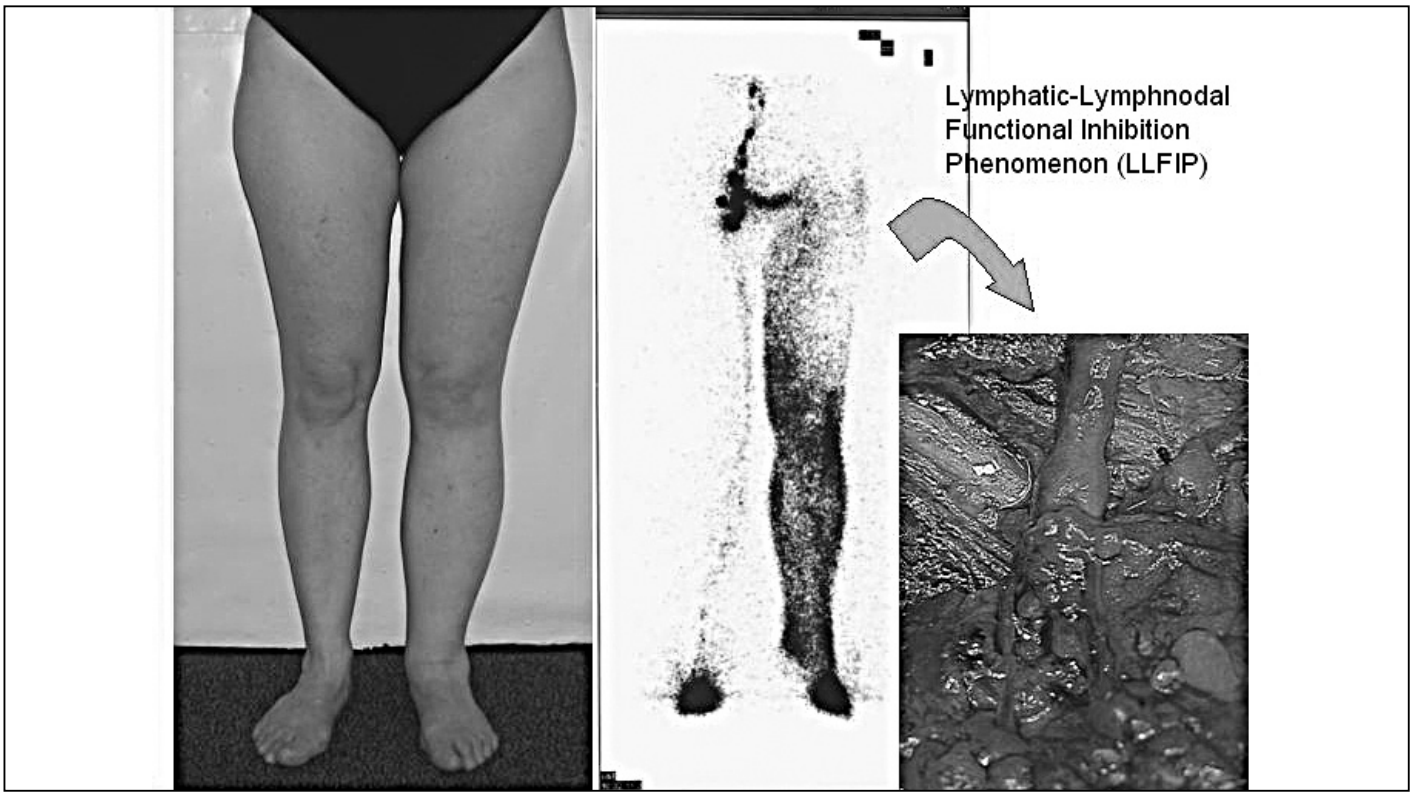


Fig. 5: Lymphoscintigraphy in a case of peripheral early stage lymphedema showing the LLFIP phenomenon.

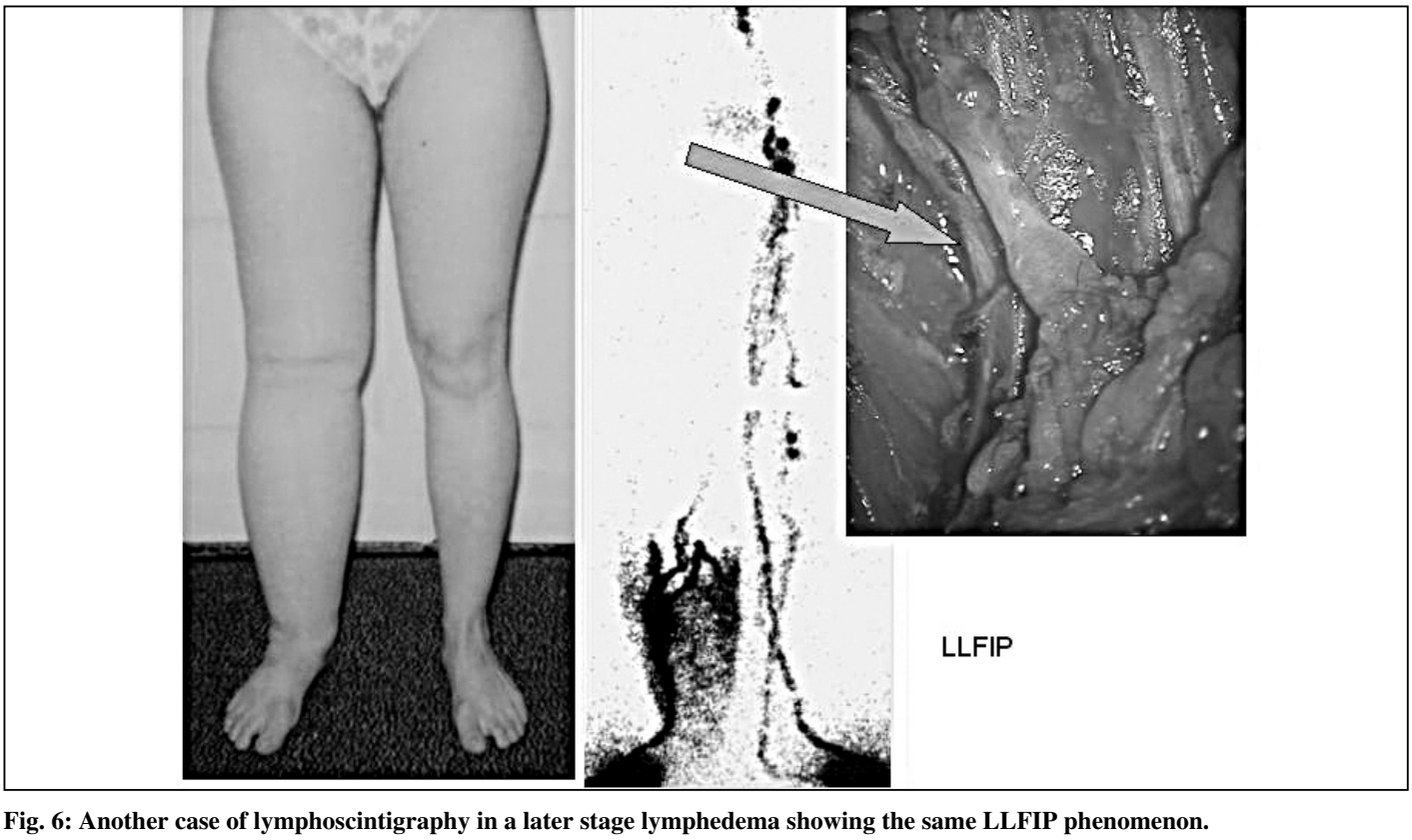


Fig. 6: Another case of lymphoscintigraphy in a later stage lymphedema showing the same LLFIP phenomenon.

DISCUSSION

Owing to these clinical observations, we suggest that in case of a complete absence of tracer progression along the limb, it is useful to inject the radiocolloid proximally at the root of the limb, a little distally to the groin or the axilla. This technical trick helps in visualizing the lymphatic structures present there and allow to properly interpret the pathology.

Finally, lymphoscintigraphy allows to assess the long term patency of microanastomoses, even over 10-15 years after microsurgical operation. Efficacy of derivative lymphatico-venous anastomoses is confirmed by the following lymphoscintigraphic patterns: 1) reduced dermal back-flow; 2) rapid clearance with the blood stream of the tracer at the site of microanastomoses, and 3) earlier tracer uptake by the liver indicative of more rapid entry into the bloodstream (Fig. 7).

As concerns reconstructive technique, lymphoscintigraphy permits to visualize lymph flow through the venous grafts thus mirroring clinical improvement. The lymphoscintigraphic patterns correlated to the efficacy of the reconstructiv technique of interpositioned vein graft are: 1) reduced dermal back flow, 2) appearance of preferential ways of lymph drainage and 3) visualization of the intralymphatic interposition autologous venous grafts (Fig. 8). It is, finally, useful to underline how there might exist some cases of "dumb lymphoscintigraphy" notwithstanding the presence of proper lymphatic-lymphnodal structures.

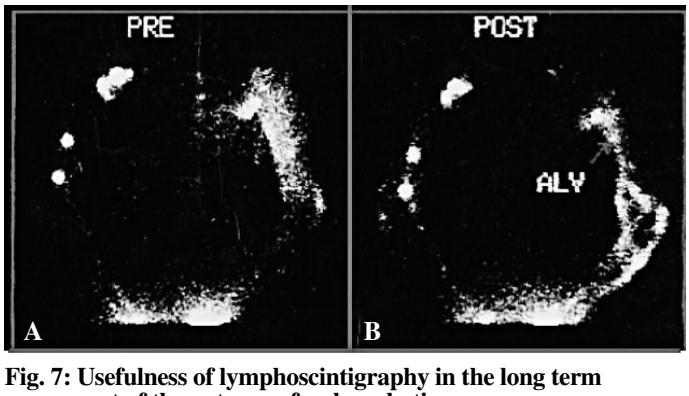


Fig. 7: Usefulness of lymphoscintigraphy in the long term assessment of the patency of ve lymphatic-venous derivatmicroanastomoses, before (A) and after (B) microsurgery.

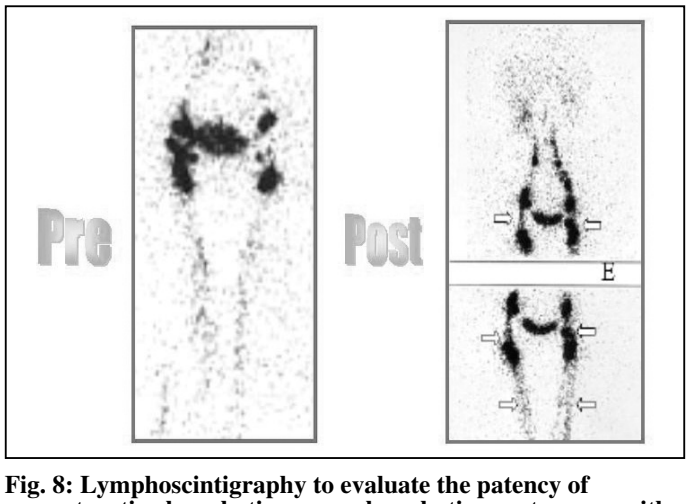


Fig. 8: Lymphoscintigraphy to evaluate the patency of reconstructive lymphatic-venous-lymphatic anastomoses with the visualization of vein grafts at long term distance from operation.

REFERENCES

1. Bellini C., Arioni C., Mazzella M., Campisi C., Taddei G., Boccardo F., Serra G.: *Lymphoscintigraphic evaluation of congenital lymphedema of the newborn.* Clin Nucl Med. 2002 May; 27(5): 383-4.
2. Biassoni P.,Campisi C.,Villa G.,Boccardo F.: *Isotopic lymphography in the diagnosis and follow-up of lymphedemas treated by microsurgery.* Lymphology 29 (Suppl): 101-105, 1996.
3. Bourgeois P., Wolter F.: *Lymphoscintigraphy demonstration of a protein loosing enteropathy.* EJLRP 1990; 18: 44-46.
4. Campisi C., Boccardo F., Tacchella M.: *The present role of isotope lymphangioscintigraphy and conventional lymphography in delineating the status of lymphatic and chylous collectors.* Lymphology 1994, 27 (Suppl), 282-285.
5. Michelini S., Campisi C., Failla A., Boccardo F.: *Proposal for stadiation of phlebolymphoedema.* Europ J Lymphol and Relat Probl 1995; 6(20): I-14.
6. Pecking A.P., Gougeon-Bertrand F.J., Floiras J.L., Garbay J.R., Banzet P., Roussé J.: *Lymphoscintigraphy. Overview of its use in the lymphatic system.* Lymphology 1998; 31 (Suppl): 343-346.
7. Witte C., McNeill G., Witte M. et al.: *Whole-body lymphangioscintigraphy: making the invisible easily visible.* Progress in Lymphology XII, Elsevier Science Publishers B.V., 1989, 123.
8. Campisi C., Davini D., Bellini C., Taddei G., Villa G., Fulcheri E., Zilli A., Da Rin E., Eretta C., Boccardo F.: *Lymphatic microsurgery for the treatment of lymphedema.* Microsurgery, 2006; 26(1): 65-9.
9. Tommasi L., Mignone A., Caralis G., Morbelli S., Curti G., Santaniello B., Boccardo F., Campisi C., Peschiera F., Dinati L., Taddei G.: *Lymphoscintigraphy in the evaluation of lymphedema in the lower limbs after abdominal surgery.* Lymphology 2004; 37 (S): 587-8.
10. Campisi C., Davini D., Bellini C., Taddei G., Villa G., Fulcheri E., Zilli A., Da Rin E., Eretta C., Boccardo F.: *Lymphatic microsurgery for the treatment of lymphedema.* Microsurgery, 2006; 26(1): 65-9.
11. Bellini C., Boccardo F., Taddei G., Mazzella M., Arioni C., Villa G., Hennekam R.C., Serra G., Campisi C.: *Diagnostic protocol for lymphoscintigraphy in newborns.* Lymphology, 2005 Mar;38(1):9-15.
12. Campisi C., Boccardo F.: *Microsurgical techniques for lymphedema treatment: derivative lymphatic-venous microsurgery.* World J Surg. 2004 Jun; 28(6): 609-13.