

WHERE DO LYMPH AND TISSUE FLUID ACCUMULATE IN LYMPHEDEMA OF THE LOWER LIMBS CAUSED BY OBLITERATION OF LYMPHATIC COLLECTORS?

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ABSTRACT

Obliteration of lymphatic collecting trunks of limbs by infective processes, trauma, oncologic surgery and irradiation bring about retention of lymph and tissue fluid in tissues. Knowledge as to where excess lymph is produced and accumulates as tissue fluid is indispensable for rational physical therapy. So far, this knowledge has been based on lymphoscintigraphic, ultrasonographic and MR images. None of these modalities provides distinct images of dilated lymphatics and fluid expanded tissue spaces in dermis, subcutis and muscles. Only anatomical dissection and histological processing of biopsy material can demonstrate the remnants of the lymphatic network and the sites of accumulation of mobile tissue fluid. We visualized and calculated the volume of the "tissue fluid and lymph" space in skin and subcutaneous tissue of foot, calf, and thigh in various stages of lymphedema, using special coloring techniques in specimens obtained during lymphatic microsurgical procedures or tissue debulking. When the collecting trunks were obliterated, lymph was present only in the subepidermal lymphatics, while mobile tissue fluid accumulated in the spontaneously formed spaces in the subcutaneous tissue, around small veins,

and in the muscular fascia. Deformation of subcutaneous tissue by free fluid led to formation of interconnecting channels. In obstructive lymphedema caused by obliteration of collectors, lymph is present mainly in subepidermal lymphatics, and the bulk of stagnant tissue fluid accumulates in subcutis between fibrous septa and fat globules as well as above and underneath muscular fascia. These observations provide useful clues for designing pneumatic devices and rational manual lymphatic massage to move stagnant tissue fluid toward the non-swollen regions.

Keywords: lymph, tissue fluid, lymphedema, histology, limbs, edema

Infections and trauma of limb skin and soft tissues evoke reactions in peripheral lymphatics and lymph nodes (1- 4). During the course of a continuing response, lymphatic structures become destroyed, tissue fluid transport toward and along lymphatics slows down, and edema of dermis, subcutaneous tissue, as well as muscular fascia and muscles gradually develops. In addition to inflammation and trauma, the iatrogenic damaging factors for lymphatics include surgery and irradiation of lymph nodes applied in cancer therapy. Subsequently, changes in tissue and collecting lymphatics similar to those

observed after infection and trauma develop (Fig. 1) (5, 6). In addition, the remaining inguinal lymph nodes likely undergo atrophy due to lack of antigenic stimulation from afferent lymph (Fig. 1). The degree of edema depends in large part on whether obstruction affects the superficial or deep or both lymphatic systems. Damage to the superficial collecting trunks is followed by edema of skin and subcutaneous tissue whereas obstruction of both drainage systems brings about a rapid and difficult to control accumulation of tissue fluid not only in superficial tissues but also under the muscular fascia and between muscle fibers.

Our understanding of the limb lymphatic network in normal physiological as well as lymphedema conditions is generally based on lymphographic or lymphoscintigraphic pictures depicting the superficial and deep systems and lymph nodes (7,8). The techniques do not allow visualization of the smaller lymphatic structures located under the epidermis where stagnant lymph accumulates. Direct lymphangiography with fluorescent tracers may be helpful delineating these minor dermal lymphatics but it is rarely used because of the special equipment requirement (9). Ultrasonography, computer-assisted tomography and magnetic resonance imaging provide pictures of tissue spaces filled up with stagnant tissue fluid, however, they do not currently show lymphatics (10-12). None of these listed methods thus provides images giving an idea how the entire "lymph and tissue fluid space" comprising interstitial space and lymphatics looks in reality. It remains difficult to imagine how tissue fluid, in the areas with obstructed main lymphatics, finds its way to the normal non-congested tissue regions and gets absorbed there. So far, only anatomical dissection and histological processing of biopsy material can visualize the tissue lymphatic network and the sites of accumulation of the excess of mobile tissue fluid.

In this study, we visualized and calculated the volume of the "tissue fluid and

lymph" space in skin and subcutaneous tissue of foot, calf and thigh in various stages of lymphedema in specimens obtained during lymphatic microsurgical procedures or tissue debulking. The observations made provide useful hints for designing pneumatic devices and rational manual lymphatic massage to move stagnant tissue fluid toward non-swollen regions.

MATERIAL AND METHODS

Tissue Specimens

Groin, calf, and foot skin and subcutaneous tissue, and inguinal lymph node specimens were obtained from 30 randomly selected patients with lower limb obstructive lymphedema stage I to IV, successively as they appeared up in our outpatient clinic at the Central Clinical Hospital Warsaw, undergoing elective lymphovenous shunt or debulking surgery. Controls were specimens of 12 patients with normal limbs operated upon for correction of fracture malunion. Fragments of inguinal lymph nodes were harvested during the lymphovenous shunt operations.

Lymphedema either developed spontaneously, after an episode of dermatitis, or following infected foot abrasion. The average duration of swelling at the time of admission was 7 ± 1 (SD) years. Sixty percent of patients experienced at least one attack of recurrent dermatolymphangiadenitis (DLA) over the last year and were treated with antibiotics. Staging of edema was based on our published classification (13). Briefly, stage 1: edema of foot, pitting subsiding after rest; stage 2: edema of foot and up to the mid-calf, only partly subsiding from foot; stage 3: non-substiting edema of foot and calf, hyperkeratosis of toe skin; and stage 4: edema of entire limb, hard foot and calf skin. Most all patients had limb lymphoscintigraphic imaging performed with ^{99}Tc labeled aggregated albumin (Nanocoll, Amersham, Switzerland). In all investigated cases, no

superficial collecting trunks could be visualized. Those in stage III and IV had MRI performed to evaluate the thickness of subcutis and its water content. Excluded were specimens from patients with acute DLA, skin ulcers, chronic venous insufficiency, limb ischemia, lipedema, and rheumatoid arthritis. The study was approved by the ethics committee of the Warsaw Medical University and the Indian Council for Medical Research and oral informed consent was obtained.

Soft Tissue Staining for Visualization of the “Lymph and Tissue Fluid Space”

Sites of accumulation of stagnant lymph and tissue fluid in the interstitial space were visualized by injecting the composite skin, subcutaneous tissue and fascia blocks with Paris blue dye in chloroform suspension (14,15). Fragments of lymph nodes were injected with the suspension under the capsule. Large particles of this dye specifically enter lymphatics but not blood vessels. They are retained in dilated free tissue spaces and color their walls. The injected tissue fragments were placed in 5% formaldehyde, were treated with increasing concentrations of ethyl alcohol, and were processed to become translucent in methyl-salicylate solution. One hundred to three hundred thick fragments were sectioned for investigated under light transmission microscopy. The surface area of colored structures was measured at x100 magnification using the Olympus Microimage software (Olympus, Japan) for determination expression as percentage of the area of the microscopic field. The longitudinal and vertical length of stained spaces was measured to calculate their volume and expressed as percentage of tissue fragment volume.

In order to prove that the stained spaces were not blood vessels, five-by-five mm thick fragments of Paris Blue injected tissues were snap frozen at -70°C and sectioned for immunohistochemical evaluation. Sections were stained with monoclonal antibodies to

lymphatic endothelial cell hyaluronan receptor LYVE 1 (R&D, Europe), FVIII-related antigen and CD31 (Dako, Glostrup, Denmark) to identify blood vascular endothelial cells.

RESULTS

Lymphoscintigraphy demonstrated in most patients lack of patent superficial and deep lymphatic collecting trunks (*Fig. 1*), and MRI displayed “honeycomb” structures especially close to the muscular fascia (*Fig. 2*). Skin, subcutaneous tissue and muscular fascia specimens obtained from these patients, stained with hematoxyline-eosin, monoclonal antibodies, and Paris Blue showed dilatation of the subepidermal lymphatics and tissue fluid spaces in the subcutaneous tissue, around small veins, and in the muscular fascia (*Fig. 3*). The dilated subepidermal lymphatic plexus could be easily discriminated from blood vessels by positive staining with LYVE1 and by their shape. This plexus could be stereoscopically visualized by intradermal injection of Paris Blue in chloroform suspension (*Fig. 4*). The venous pattern of the same skin region looked strikingly different from the lymphatic pattern (*Fig. 4*). During the course of lymphedema, the subepidermal plexus underwent gradual destruction, and its deeper vessels become obstructed. In order to analyze where exactly tissue fluid accumulates, three anatomical regions – dermis, subcutaneous tissue, and muscular fascia – were investigated. For the most part, stagnant tissue fluid was found accumulating in the deepest layers of subcutaneous tissue composed of fibrous and fat tissue. Excess tissue fluid brought about deformation of tissue structures leading to formation of irregularly shaped channels (*Figs. 5,6*). Their walls were not lined by lymphatic endothelial cells and did not stain for LYVE 1. With progression of lymphedema, the subcutaneous space becomes richer in fibrous structures, newly formed channels closed down, and

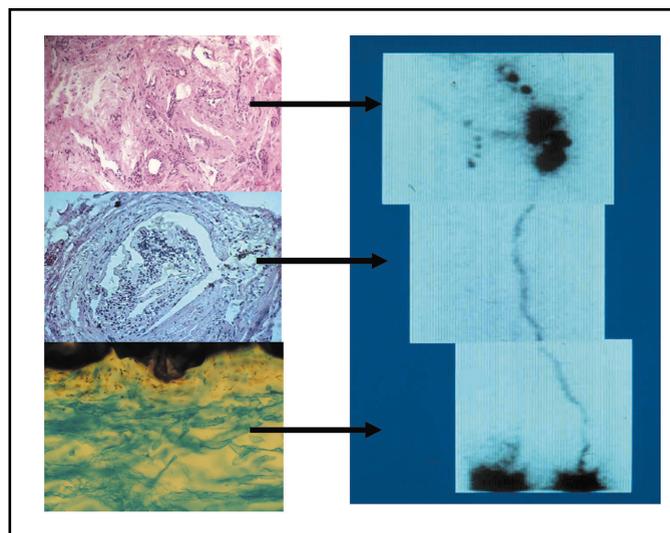


Fig. 1. Lymphoscintigraphic image of damaged lymphatics and nodes and histological pictures of biopsy of these structures in obstructive lymphedema. Right panel: A lymphoscintigram of lower limbs in a patient after hysterectomy because of cancer and removal of iliac lymph nodes. The tracer injected into the toe webspace is poorly absorbed, and it remains in the dilated superficial lymphatic plexus of the right foot. No collecting trunks are visible, and small solitary lymph nodes are seen in the inguinal area. Left-top: Histological picture of the biopsied node shows fibrosis and irregularly shaped lymph channels (mag. x200). Left-middle: A collecting trunk almost totally obliterated, and filled with a clot of mononuclear infiltrates (H-E staining, mag. x200). Left-bottom: an irregular network of subepidermal lymphatics (Paris Blue staining, mag. x100)

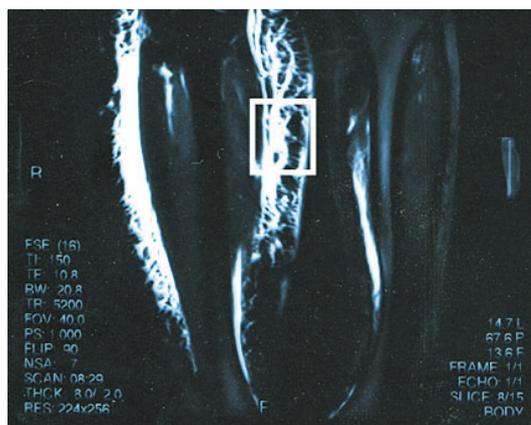


Fig. 2. Magnetic resonance image of lower limb obstructive lymphedema stage III. Thickened skin and wide layer of subcutis showing a honeycomb appearance. Biopsies were taken from this region (frame) for histological evaluation of spontaneously formed "tissue channels."

fluid accumulated in narrow spaces between collagen bundles (Fig. 6). Mobile tissue fluid could also be found in the perivascular areas

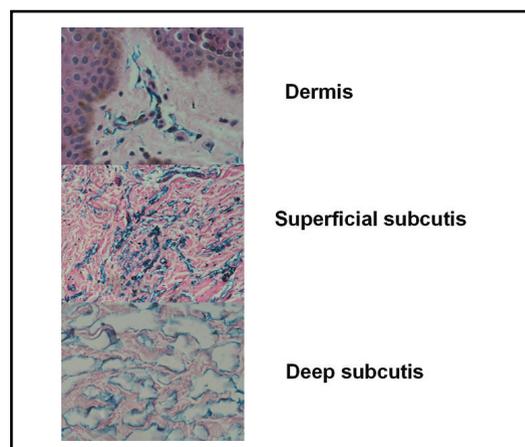


Fig. 3. Histological picture of calf epidermis, dermis, and subcutaneous tissue in obstructive lymphedema stage III. Specimens stained with Paris Blue and H-E. The thick epidermis is composed of 10-15 layers of keratinocytes. Bluish-stained minor structures in the papillary dermis are multiple small dilated subepidermal lymphatics. In the subcutaneous tissue, bluish-stained wide spaces filled with fluid are seen. Deeper in the subcutis, these spaces become larger (mag. x200).

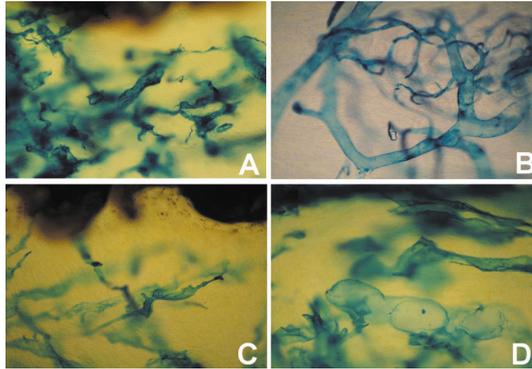


Fig. 4. Subepidermal lymphatics and veins. A,B: Lymphatics and veins from calf skin of the same patient in Fig. 2 stained by intravascular injection of Paris Blue. A: Partly obliterated, partly dilated subepidermal lymphatics. B: Retrograde injection of the dye allowed visualization of veins forming a network of small vessels merging with larger vessels. Venous architecture is shown to differentiate lymphatics from veins. C,D: Dilated groin skin subepidermal lymphatics stained with Paris Blue in a patient with lymphedema stage IV. Epidermis (C) and papillary dermis (D) display a network of still patent lymphatics with irregular shape. Some lymphatics are obliterated (C), others dilated (D). The stained area occupies 24% (C) and 38% (D) of the surface and 19% (C) and 30% (D) of the specimen volume (mag. x100).

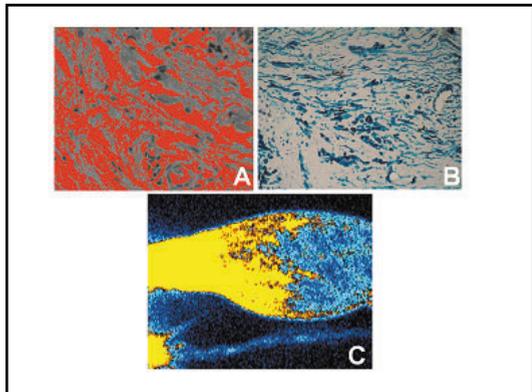


Fig. 6. Calf subcutaneous tissue in lymphedema stage IV. A. A computer image of the surface occupied by free tissue fluid (red stained) (Micro-image, Olympus, Japan). B. Muscular fascia in calf lymphedema (same patient as on Fig. 2). Multiple irregular collagen bundles separated by free spaces filled with fluid. Many of these stain bluish with Paris Blue. H-E, x100. C. A lymphoscintigram of subcutaneous tissue depicting tissue fluid channels shown at part A.

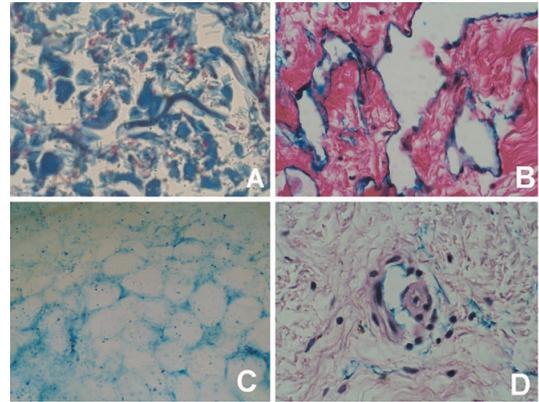


Fig. 5. Calf subcutaneous tissue at the border with muscular fascia (same patient as on Fig. 2). A. Large spaces between collagen bundles are dilated artificial tissue spaces. B. Trichrome staining shows the bluish stained walls of tissue fluid-filled spaces. They are not lined by lymphatic marker-stained endothelial cells (LYVE1 – negative) (Paris Blue staining, mag. x100). C. Fluid accumulates around the fat globules (Paris Blue + H-E stain, mag. x400). D. A bluish stained space around the vein formed by stagnant fluid (Paris Blue staining, mag. x100)

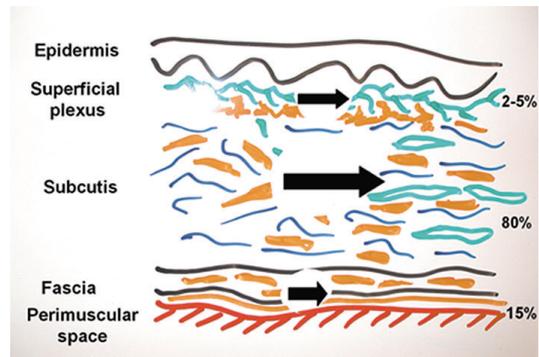


Fig. 7. Schematic presentation of the site of accumulation of lymph and free tissue fluid. The figures denote the calculated percent of total lymph and tissue fluid volume in the skin, subcutis, and fascia. Arrows show the direction of flow during massage.

(Fig. 5). Fluid accumulating in the thickened fascia formed multiple narrow longitudinal fascia channels (Fig. 6).

Quantitative evaluation of the surface and volume of dilated subepidermal lymphatics and spontaneously formed tissue spaces revealed that up to 60% of tissue

volume is occupied by stagnant lymph and tissue fluid. Inguinal lymph nodes revealed obliterated lymphatic sinuses. Their endothelial cells did not stain with antibodies against LYVE1. No perinodal accumulation of fluid was seen.

DISCUSSION

Knowledge about where excessively produced lymph and tissue fluid accumulate is indispensable for rational physical therapy. So far, this knowledge has been based on lymphoscintigraphic, ultrasonographic, and MR images. None of these modalities provides distinct images of dilated lymphatics and expanded tissue spaces in the dermis, subcutis, and muscles. Only anatomical dissection and histological processing of biopsy material can show the remaining non-obstructed lymphatic network and the sites of accumulation of mobile tissue fluid. Our studies are the first in the literature to visualize the sites of accumulation of stagnant lymph and tissue fluid and provide data on their volumes. We found that in patients with obstructed limb lymphatic collectors, lymph was present only in the subepidermal lymphatics whereas mobile tissue fluid accumulated in the spontaneously formed spaces in the subcutaneous tissue, around small veins, and in the muscular fascia. Foot, calf, and thigh tissue contained similar volumes of fluid reaching on the average 50% of the total tissue volume (*Fig. 7*).

The most superficial layer accumulating fluid was the subepidermal lymphatic plexus occupying a 200-300 um thick papillary and reticular dermis. However, the volume of fluid in this plexus is negligible compared with the volume of the subcutaneous tissue fluid and does not exceed 2-3% of total tissue fluid retained in soft tissues (personal observations). Some vessels of the subepidermal plexus pierced the epidermis and formed small superficial blisters. The reason that subepidermal lymphatics remain patent while the collecting trunks are obstructed is

unclear. Progressive fibrosis of dermis brought about gradual obliteration of the plexus.

The bulk of mobile tissue fluid accumulated in the subcutaneous tissue forming artificial partially interconnected spaces. These spaces were located between fat globules, fibrous bundles, and around small veins. Formation of large perivascular spaces containing tissue fluid could be explained by the presence of lax connective tissue in these regions, its high compliance, and subsequently low resistance to flow.

A new finding was formation of tissue fluid channels around and in the hypertrophic muscular fascia of the calf. These were narrow longitudinal spaces between the fascial fibrous elements. The hydraulic conductivity of these structures would be expected to be high because of linear positioning of fibers.

The inguinal lymph nodes revealed major changes in the sinuses including obliteration and formation of blind spaces and depletion of lymphoid elements. High resistance to lymph flow in the fibrotic nodes may be a factor causing stagnation of lymph in the rudimentary patent afferent lymphatics. There was no detectable accumulation of tissue fluid in the perinodal space.

The volume of fluid accumulating in the tissue spaces and calculated from densitometric readings of the stained tissues reached 50 to 60% of tissue volume. Measuring tissue fluid content and its topographical distribution may be done using non-invasive methods such as MRI. However, the resolution power of MR is still too low to display minor lymphatics, small tissue fluid "lakes," and thin fluid layers above and below fascia. Fumiere et al found that normal subcutaneous septa are seen as hyperechogenic lines by ultrasound and hyposignal lines in MRI, and that hyperechogenic subcutis in US can be due to interlobular and intralobular water accumulation and/or to interlobular and intralobular fibrosis (16). They advised multiple imaging modalities to precisely delineate the nature of tissue water accumulation in lymphedema. Idy et al demonstrated

water retention diffusely spreading over the entire dermis and fluid retention located in the interlobular spacing and beside the superficial fascia. Within the subcutis, they identified superficial fat lobules, but not much fluid accumulation (11). However, these images did not precisely depict the location and shape of tissue fluid formed spaces and the structure of their walls. Our observations, based on studies of harvested tissue, supplement the knowledge obtained from the noninvasive imaging on the topography of mobile fluid accumulation and shape of channels in the edema-deformed tissue. Knowledge about where the tissue fluid is located should be useful for designing pneumatic devices for limb massage as well as for rational manual lymphatic drainage. The data obtained provide hints on how to design the shape of sleeves and where to press manually in order to effectively move fluid toward non-swollen regions.

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