

## MORPHOLOGIC AND FUNCTIONAL CHANGES OF THE MICROLYMPHATIC NETWORK IN PATIENTS WITH ADVANCING STAGES OF PRIMARY LYMPHEDEMA

C. Allegra, R. Sarcinella, M. Bartolo, Jr.

Department of Angiology, S. Giovanni Hospital, Rome, Italy

### ABSTRACT

*Using fluorescent microlymphography, we examined the morphology and functional characteristics of the microlymph-vascular network in 36 subjects including 9 patients with "compressible" lymphedema (Group I), 14 with minimally compressible lymphedema (Group II), 9 with noncompressible lymphedema (Group III), and 4 healthy individuals.*

*As lymphedema progressed from early to advanced stages (Group I-III), an increasingly greater lymphatic capillary density and diameter were depicted and eventually fibrosis/sclerosis with lymphangiectasia, fragmentation and a gradual decrease in the number of microlymphatics. Concomitantly, there was a prolongation in transport and disappearance of fluorescent dye and a progressive increase in endolymphatic and interstitial hydrostatic pressures.*

Microlymphography was first performed using Dextran 40,000 but its relatively small molecular size yielded confusing lymphatic transport data as the marker was partially absorbed by blood capillaries and excreted by the kidneys. Lymphoscintigraphy using radiolabeled (<sup>99</sup>Tc) Dextran 110,000 demonstrated uptake of the radiotracer by macrophages and its insolubility in interstitial fluid. Using computer assisted dynamic imaging, serial blood sampling in 13 dogs

confirmed that the radiotracer was cleared only by lymphatics from the interstitial injection site with a half-time of 31.5 minutes (1). In experimental peripheral lymphedema, radiotracer migration from the edematous hindlimb was markedly delayed and the image resembled the appearance seen with direct contrast lymphography. Although findings in humans are still relatively few, similar data have been obtained.

Intaglietta et al in 1971 (2) determined the pressure inside a biological fluid using 7-9  $\mu$  glass microneedles and a Servo-Nulling Pressure System. In 1980, Wiig et al (3) found tissue pressure in resting rats to be slightly below atmosphere using this technique. Controversy has persisted, however, about the true tissue pressure and the interstitial protein concentration under normal conditions and in disease states. Expanding upon the Starling microcirculatory forces that are responsible for the partition of extracellular fluid between plasma and the interstitium, Guyton (4) identified several key factors that limit edema formation. These include an increase in tissue hydrostatic pressure, a decrease in plasma oncotic pressure and an increase in lymph flow. The absolute values, however, change and are dramatically effected by skeletal muscle contraction, external compression, respiration (5-7), impaired lymphatic pumping (lymphangion contractility), and pathologically altered tissues in a wide

**TABLE 1**  
**Classification of Primary Lymphedema Using Fluorescent**  
**Microlymphography (see Ref. 6)**

Stage	Findings
I	Lack of initial lymphatics in the presence of deep collectors which are partially structurally abnormal and partially dilated.
II	Initial lymphatics present (normal caliber, or dilated, or increased in number) with hypoplasia or aplasia of peripheral lymphatic collectors.
III	Dilation of initial lymphatics and peripheral collectors which sometimes are increased in number.
IV	Reduced or absent initial lymphatics and obliterated peripheral lymphatic collectors.

variety of diseases (8). In general, edema formation is counterbalanced either by greater lymph flow or proteolysis of macromolecules sequestered in tissue spaces (9,10) especially in primary lymphedema where infiltration by tissue macrophages is striking.

Hypoplasia of lymphatic collectors (i.e., primary lymphedema) is typically associated with an increase in tissue hydrostatic pressure. In this disorder, initial lymphatics (capillaries) act as passive conduits at first but with mechanical distortion of their anchoring filaments, intraluminal valves, and endothelial lining, a secondary accumulation of tissue protein and cellular elements rich in cytokines may obliterate or cause them to take on pump-like capacity. Primary lymphedema, therefore, at its inception is deceptive because at first superficial skin lymphatics may adjust and handle the tissue protein and liquid load in conjunction with a reduced lymphatic collector capacity. In time, however, these "compensatory" mechanisms are overworked or become disturbed and edema worsens and hardens.

In both primary and secondary lymphedema, the initial lymph cutaneous network is abnormal. Bollinger et al in 1981 (11) examined initial lymphatics in healthy subjects and in patients with lymph stasis using fluorescent microlymphography. Whereas they identified 4 types of disturbed microlymphatic topography (*Table 1*), their classification failed to distinguish among different kinds of lymphatic insufficiency or its various evolutionary stages. For example, the classification does not consider transport dynamics and pressure differentials in the microlymphatics or the interstitium, nor does it try to correlate the clinical presentation with microlymphatic dysfunction.

The aims of this research were: a) to correlate the stages of lymphedema (12) with microlymphographic dysfunction; b) to examine the lymph transport and pressure dynamics in the microlymphatics and adjacent tissues; c) to assess the disappearance rate of the "stained" microlymphatic network as an indicator of incompetence of lymphatic collectors (13-16).

## MATERIALS AND METHODS

Thirty-two patients with primary lymphedema of the lower extremities were examined and divided into 3 groups according to the clinical stage (12).

Group I – 9 patients with “compressible” edema (8 women and 1 man; age  $35 \pm 4$  years) (mean  $\pm$  SD).

Group II – 14 patients with moderately compressible edema (9 women and 5 men; age  $40 \pm 2$  years).

Group III – 9 patients with noncompressible edema (6 women and 3 men; age  $44 \pm 5$  years).

Group IV – 4 subjects without leg edema and otherwise healthy (age  $34 \pm 4$  years).

Microlymphography was performed using a moving arm with a fluorescence videomicroscope (Wild Leitz). Using magnification (1x) the microlymphatic network was visualized after a subepidermal injection of 0.01 FITC Dextran 150,000 (25%) 5 cm above the medial malleolus under microscopic control. Photomicrographs were filmed using videocamera (Ikegami ITC-410) and transformed into video signals to a digital videorecorder (Sony SLV-415) and simultaneously depicted on 2 or more monitors. Recordings lasted for at least 15 minutes.

From these images we analyzed:

- a. Morphology of the lymphatic network (13-18).
- b. Mean diameter of the microlymphatics using morphometric computerized elaboration and estimated (in  $\mu$ ) choosing among 10 of the best stained meshes (13-18).
- c. Velocity of fluorescence staining or the time needed to visualize (i.e., stain) the microlymphatic network from the time of intradermal inoculation (17).
- d. “Permanency” or the amount of time that the microlymphatic remains stained.
- e. “Extension” or the distance from the inoculation area to depiction of the microlymphatic network (17).
- f. Diffusion or the loss of fluorescent dye into perilymphatic tissues (transudation or dissolution of the microlymphatic) (17).

Parameters c-f are directly correlated with opening of lymphatic collectors, degree of lymphatic hypoplasia, and the integrity of the lymphatic vessel in response to increased cutaneous pressure.

The Servo-Nulling System apparatus (Mod 5A) consists of a pressure transducer (Mod 915), a video signal control (515), and a micromanipulator. With 3.2x magnification, we measured (5):

- a. Intraluminal lymphatic pressure by introducing a 7-9  $\mu$ m microneedle probe on the micromanipulator into the most densely stained lymphatic and connected it to the Mod 5A to yield an intraluminal pressure for at least 1 minute. Computer analysis thereafter provided the range and mean of the intraluminal lymphatic pressure recorded.
- b. By manipulating the microneedles into the adjacent tissue, interstitial pressure was determined and used as a reference for intralymphatic pressure readings including initial lymphatics highlighted by following the diffusion of fluorescent Dextran.

## RESULTS

Dermal microlymphatics were displayed in all groups. *Tables 2 and 3* demonstrate the data obtained in each group of patients compared with healthy subjects (controls). Computerized analysis provided the mean  $\pm$  data for each parameter tested (6.06 SAS, VAX/UMS).

### *Group I (Compressible Primary Lymphedema)*

Compared with healthy subjects, initial lymphatics were greater in number ( $p < 0.001$ ), enlarged ( $p < 0.001$ ) and organized into a more extensive network ( $p < 0.001$ ). Moreover, after injection of fluorescent Dextran, the microlymphatic network was rapidly depicted similar to controls, but diffusion into the surrounding tissue was slower ( $p < 0.001$ ) and persistence of microlymphatic staining was prolonged ( $p < 0.001$ ). Endolymphatic pressure was also increased ( $p < 0.002$ ) as was interstitial pressure ( $p < 0.001$ ).

**TABLE 2**  
**Microlymphatic Network Changes in Primary Lymphedema Using Fluorescence**  
**Microlymphography (see text for details)**

Parameters	Group I	Group II	Group III	Control
Initial Lymphatics (#)*	32±5	30.6±8.23	10.6±7.2	7±5.03
Diameter (μ)	121±48	166±51	142±47	54±1
Velocity (sec)	1.5±0.6	120±42	250±39	1±0.3
Diffusion (μ)	13.83±7	21±11	32±16	3.07±1.1
Extension (μm)	40±8.7	30±2.5	18±1	6±2
Permanence (min)	8±3.5	20±4.8	17±5.9	0.48±1.7
* $\bar{x} \pm SD$				

**TABLE 3**  
**Microdynamic Pressures in Primary Lymphedema**

Pressure (mmHg)	Group I	Group II	Group III	Control
EP	8.2±1.7	10.03±2.1	11±1.5	4.19±1.9
IP	4.31±1.98	5.18±1.48	7.2±1.9	0.65±1
EP=endolymphatic pressure; IP=interstitial pressure				

*Group II (Moderately Compressible Primary Lymphedema)*

Compared with healthy subjects, the microlymphatic network was again greater ( $p<0.001$ ), more enlarged ( $p<0.001$ ), but with slower diffusion ( $p<0.001$ ), reduced velocity of microlymphatic staining ( $p<0.001$ ), and greater permanence of staining ( $p<0.001$ ).

Although the microlymphatic network was more extensive than in controls ( $p<0.001$ ), it was less extensive than in Group

I. Both endolymphatic and interstitial pressure were higher than in controls and higher than in Group I ( $p<0.001$  and  $p<0.002$ , respectively).

*Group III (Noncompressible Primary Lymphedema)*

At this more advanced stage, microlymphography became more difficult. Fewer lymphatic vessels were depicted ( $p<0.001$ ). Moreover, they appeared tortuous, sometimes

damaged or obstructed albeit dilated ( $p < 0.001$ ), but, nonetheless, still more extensive than controls ( $p < 0.001$ ), yet less than Group I and II and quite irregular. Staining was much slower ( $p < 0.001$ ) and more retarded than in Groups I and II. Diffusion was slower and permanence greater than in controls ( $p < 0.001$ ) and similar or worse to Groups I and II.

Endolymphatic and interstitial pressure were both significantly increased over controls as well as greater than Group I and II ( $p < 0.001$  and  $< 0.002$ , respectively).

### DISCUSSION

Intradermal injection of 25% isothiocyanate with fluorescent Dextran 150,000 at low dose (0.01 ml) provides good display of lymphatic capillaries without allergic reactions and is based on the fact that an elevation in tissue hydrostatic pressure promotes lymph transport (19). In healthy subjects, tissue hydrostatic pressure or the prelymphatic pathways is slightly below atmosphere. As the tiny lymphatics or initial lymphatics are normally collapsed they, too, have a slight negative intraluminal pressure. With increased tissue pressure, initial lymphatics fill with fluid and a positive intraluminal pressure develops. As the endolymphatic pressure rises, lymph transport assumes a positive velocity which increases in proportion to the tissue hydrostatic pressure and endolymphatic pressure (19,20). Gradually, a pressure gradient develops between prelymphatics and initial lymphatics for gradual filling and flow of fluid. A slight adjustment in the tissue oncotic pressure also concomitantly occurs (7,19-21). When increased tissue hydrostatic pressure exceeds the capacity of lymphatics to remove surplus tissue fluid, the excess liquid accumulates in the interstitium to form edema (12,20,22). The accumulation of plasma protein in the interstitium increases the local osmotic pressure promoting collapse of the initial and terminal lymphatics. This

combination of events culminates in lymphedema. Primary lymphedema from developmental hypoplasia of subdermal lymphatic collectors generates an overload of fluid and protein in the interstitium due to insufficient drainage by deep lymphatics. Our microlymphographic findings document the gradual evolution of microlymphatic changes as primary lymphedema progresses from Stage I-Stage III. In early compressible lymphedema, an increase in cutaneous microlymphatics occurs with slight prolongation of microlymphatic staining (permanence). This staining fades locally and colors more proximal lymphatic vessels. In conjunction with minimally increased diffusion of the fluorescent dye, it suggests the existence of lymphatic-lymphatic anastomoses. With less compressible lymphedema (Group II), the interstitial protein content tends to rise (10,23,24). At this stage, the microlymphatic network holds the fluorescent dye for a more prolonged period, a finding that reflects restricted drainage into deeper lymphatics. The accumulation of tissue macrophages (8,23,24) probably aggravates the restriction to lymph transport.

In the more advanced or noncompressible stage of lymphedema (Group III) where subcutaneous fibrosis is more prominent, the microlymphatic network is reduced but dilated as lymph stagnates. It remains unclear, however, whether lymphatics reconstitute new drainage conduits or are obliterated and compressed by the surrounding sclerosis (24).

As primary lymphedema advances, the endolymphatic pressure rises in proportion to the ability of the superficial lymphatic network to dissipate the rising interstitial pressure. Thus, both pressure measurements are notably increased compared with normal subjects and the degree of rise is proportional to the stage of lymphedema (10,20). This fact is supported by the microlymphographic findings in the early stage (Group I) where dye refluxes outside the stained network. Thus, fluorescence is seen in deep lymphatics

via hypoplastic or insufficient collectors. This phenomenon has also been described with the "blue dye test" and with indirect lymphography (25,26). The disappearance or lack of diffusion in the advanced stage of primary lymphedema reflects the inability of the lymphatic microcirculation to offset the elevated endolymphatic and interstitial hydrostatic pressure.

These findings, taken together, provide direct information about the structural and functional derangements of the superficial lymph vascular system and local tissue pressure dynamics as lymph stasis progressively worsens. Although limited by a restricted area of view, fluorescent microlymphography demonstrates that the derangements in the deep lymphatics and collectors (macrolymphatic system) do not necessarily parallel the morphologic and physiologic changes in the microlymphatic network.

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**Prof. C. Allegra, M.D.**  
**Chief, Department of Angiology**  
**S. Giovanni Hospital**  
**Roma, Italy**