

LYMPHATIC CLEARANCE OF THE HUMAN SKIN IN PATIENTS WITH ACUTE DEEP VEIN THROMBOSIS USING A NOVEL FLUORESCENT TECHNIQUE

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ABSTRACT

The purpose of this study was to investigate lymphatic clearance of the human skin in patients with acute deep thrombosis of the femoral vein. In 13 patients with deep vein thrombosis and no other cause for swelling of the limbs, lymphatic clearance of the skin at the foot was measured. Ten microliters of fluorescein isothiocyanate-dextran 150,000 were injected intradermally and the fluorescent light intensity of the deposit measured 10 min and 24 hours after injection by window densitometry. In addition, intralymphatic pressure was measured by the servo-nulling system. The results were compared with a sex- and age-matched control group. Fluorescent light intensity decreased by 23.8 ± 12.3 arbitrary units or by a factor of 1.8 ± 0.5 in patients with DVT after 24 hours, which was significantly less than in healthy controls (33.7 ± 8.9 arbitrary units or by factor 5.0 ± 4.1 , $p < 0.013$). Intralymphatic pressure was not different between the two groups. These results indicate that lymphatic clearance is significantly reduced in the acute phase of deep venous thrombosis.

Keywords: microlymphography, lymphatic clearance, lymphatic pressure, fluorescence densitometry, deep venous thrombosis

The lymphatic system functions in draining the lymph fluid from the interstitium back to the blood vessels in physiological quantities, and a diminished lymphatic clearance of ultrafiltrate results in accumulation of interstitial fluid and hence in edema formation. It is well known that lymphatic function is decreased in patients with chronic venous disease (1-3), and it has been demonstrated in chronic venous insufficiency (CVI) that severe microangiopathy of the initial lymphatics of the skin is present (4-6). However, only very little is known about lymphatic function in the acute phase of deep venous thrombosis (DVT) of the leg. The purpose of this study was to investigate the lymphatic clearance of the human skin in patients with acute DVT of the leg. The rate of removal of large molecules such as proteins and large sugars that are cleared exclusively by lymph vessels provides an index of the lymphatic function of the skin (7). In addition to the isotope clearance technique, which has been widely used in subcutaneous tissue (8,9), a new and safe method using a fluorescent tracer to assess the lymphatic clearance in normal human skin has been described and the reliability and reproducibility of this method has been demonstrated (10). This fluorescence microlymphography allows the visualization of the initial lymphatics and

measurements of indirect parameters of lymphatic function such as extension of the dye in the superficial network (11) as well as lymphatic pressure as a direct parameter (12,13).

SUBJECTS AND METHODS

Thirteen patients (5 male, mean age 60.2 ± 19.6 years) with acute DVT of the femoral vein and no other cause for swelling were included in the study. All study subjects gave informed consent and the study was approved by the Ethical Committee of the University Hospital Zurich. DVT was diagnosed by duplex ultrasound and only those patients in whom the history of swelling or pain was not older than 24 hours were included. Patients with previous deep vein thrombosis or any clinical signs of CVI, lymphatic disease, or previous history of leg swelling were excluded. In all patients, treatment of DVT was initiated with low molecular weight heparin (200IU/kg body weight per day) and compression therapy on an outpatient basis. The first measurement was taken immediately after the diagnosis was made and before compression therapy was started. Because we wanted a comparison to normal limbs, the contralateral limb was not used due to possible physical effects (reduced physical activity, elevation of legs, etc.) by the patient with an acute DVT and, therefore, the results were compared with a sex- and age-matched control group.

Lymphatic Clearance

Clearance was measured using fluorescence microlymphography, which has been described previously in detail (11) as well as the recently described technique of fluorescence densitometry (10). The exactly reproducible amount of 10 μ l of a sterilized 25% solution of FITC-dextran (150,000 molecular weight; Sigma Chemical, St. Louis, Mo., USA), a fluorescent large sugar which is exclusively drained by the lymphatic system,

is injected into the subepidermal layer of the skin. The superficial lymphatic network of the skin is then visualized by a fluorescence video microscopy system. The microscope is equipped with 1.0/0.04, 2.5/0.08, 6.3/0.20 and 10/0.25 planar objectives (Leica), which allow a magnification of x24, x62, x165 and x240, respectively, on the monitor. The fluorescence excitation filter excites at 450-490 nm, and the barrier filter blocks at 515 nm and above.

The subjects were placed in supine position, the skin marked with a waterproof pen for locating follow-up investigations, and the measurements were performed after a resting time of at least 10 min in a temperature-controlled room (22-24°C). The dye was injected in the dorsum of the foot and after a period of 10 min the dye deposit was observed for 1 min and stored on videotape. The planar objective 1.0/0.04 (magnification x24) was used for this entire observation. Twenty-four hours later, the imaging was repeated. All subjects were asked to resume their daily activities but not to perform sports between measurements.

On-Line Measurements

The spreading of the dye deposit (proximal-distal and medial-lateral) was measured exactly 10 min after dye injection using a length scale. This measurement was repeated at follow-up examinations. The maximal intralymphatic extension of the dye apart from the depot was measured exactly 10 min after dye injection, again using a ruler.

Off-Line Measurements

The densitometric off-line measurements was performed using the CapiFlow software (Electrum 232, Kista, Sweden) and a personal computer (Compaq Deskpro 4/66i, Compaq Computer Corporation, Houston, TX, USA). Three rectangles of 1.0 x 0.7 mm (0.7 mm²) were used as windows for the densitometry analysis and placed over the dye deposit and the reference area 30mm from the dye

injection to assess the natural (control) light intensity of the skin, which was subsequently subtracted from the fluorescent value. The windows were placed at exactly the same position at follow-up examinations 24 hours later. Light intensity was measured in digital arbitrary units (AU) (0-255) during a time period of 1 min, 10 min after dye injection and at follow-up examinations.

Intralymphatic Pressure Measurements

Lymphatic capillary pressure (LCP) was measured by using the servo-nulling system (Model 5A; IPM, San Diego, Calif., USA) at day one. The method of LCP measurement has previously been reported in detail (12). Briefly, a glass micropipette (tip diameter of 7-9 mm) was inserted into a well-delineated lymphatic capillary by means of a micro-manipulator (Leica, Glattbrugg, Switzerland) to obtain measurements. LCP was recorded in two lymphatic capillaries and only measurements of at least 20 s duration were used for calculation. Fluorescence microscopy provided visual control.

Statistical Analysis

Analyses were performed with the statistical software package Stat View 5.0. (Abacus Concepts, Inc., Berkeley, CA, USA). Variables are reported as mean \pm SD. Comparison between groups was evaluated by means of Mann-Whitney U Test, and Wilcoxon signed rank test was used for intragroup differences. Significance was defined as $p < 0.05$.

RESULTS

There was no difference in the extension of the original dye deposit in healthy controls (6.3 \pm 1.7mm) and in patients with acute DVT (6.4 \pm 1.1mm) at day one. At day 2, the dye deposit extended to 13.0 \pm 2.4mm in controls and 11.3 \pm 3.0mm in patients ($p > 0.05$). In both groups there was a significant increase of the

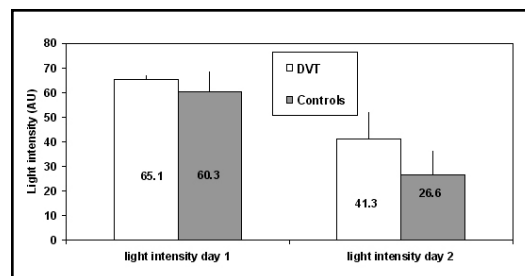


Fig. 1: Fluorescence light intensity decreases significantly more in healthy controls than in patients with DVT from day 1 to day 2 ($p = 0.0017$).

deposit from day one to day two ($p < 0.05$) but no difference between the two groups was seen. No difference was found in the maximal intralymphatic extension of the dye in the two groups (6.1 \pm 3.9mm vs 5.2 \pm 4.0mm) 10 min after dye injection. In patients and controls, no lymphatics were seen after 24 hours.

The values of the fluorescence densitometry at day one were not different between the groups (60.3 \pm 8.4 AU vs 65.1 \pm 2.0 AU) but they were significantly lower in controls (26.6 \pm 10.1 AU) than in DVT (41.3 \pm 10.9 AU) at the second measurement 24 hours later ($p = 0.0017$, *Fig. 1*). This corresponds to a significantly higher decrease factor of 5.0 \pm 4.1 times in controls vs 1.8 \pm 0.5 times in DVT ($p = 0.013$).

LCP was 7.5 \pm 3.1 mmHg in controls and 10.7 \pm 5.5 mmHg in DVT at day one. There was no difference between the two study groups ($p > 0.05$).

DISCUSSION

In the present study lymphatic function in patients with acute DVT has been evaluated using a novel technique assessing lymphatic clearance with a fluorescent tracer. This recently described method allows measurement of lymphatic clearance from skin with minimal invasion and with the advantage of visualization of the initial lymphatics for additional morphological and functional studies (10). It is well known that

in chronic venous disease, lymphatic function is decreased and this might be an important factor in the pathology of venous ulcers (1,3,6,14-17). In venous thrombosis, microvascular pressure rises leading to increased filtration. If the lymphatic system fails to remove the additional amount of interstitial fluid, edema develops. Early in the process, lymphatic vessels compensate for the additional tissue fluid by dilating and later by increasing the number of lymphatics. In the late phase of severe CVI, lymphatic vessels become insufficient, and, in addition, severe lymphatic microangiopathy develops leading to the well known skin changes (1,3,18).

However, very little is known about the influence of an acute increase of the venous pressure on lymph dynamics. In an experimental study it has been reported that during venous congestion lymph flow decreases by about 50% (19). The first publication investigating the effect of DVT on lymphatic function in an animal model showed that in the initial phase of DVT, lymph flow decreases (20). This may be due to the involvement of the subfascial lymphatics in the inflammatory reaction around an area of thrombosis. We found that patients with an acute DVT of the femoro-popliteal segment have a significant decrease in lymphatic clearance of the skin of the affected limb. It has been shown in postthrombotic syndrome that augmented prefascial lymph transport is present, which serves to compensate for decreased subfascial lymph transport (3). This collateral lymph drainage via the epifascial, subcutaneous and skin vessels that develops when the normal routes are damaged, as during deep vein thrombosis, might not be sufficient in the very early stage of DVT. However, it takes days to weeks for edema resolution after deep vein thrombosis. The explanation might be a delayed compensatory increase in lymph clearance, which has not been assessed in our study. Therefore, our results of decreased lymphatic clearance of the skin in the very acute phase of DVT are not in conflict with the aforementioned findings of increased

epifascial lymph drainage postthrombotic syndrome, and the difference may only be due to timing of the measurements.

LCP did not increase significantly in patients with DVT in our study. However, a tendency towards higher pressure values was observed. Because we included only patients with DVT of the femoro-popliteal veins, it may be that LCP would rise in patients with DVT of both the femoral and iliac veins. The increase in the deposit area of the dye after 24 hours was not different in the two groups indicating that the decrease in light intensity is not due to different diffusion in the tissue but reflects lymphatic clearance.

In conclusion, this study using a novel technique with fluorescent tracers is the first to demonstrate that lymphatic clearance of the skin is reduced in the acute phase of DVT.

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