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Lymphatic microangiopathy of the skin in systemic sclerosis

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Abstract

Methods. The cutaneous capillary lymphatic system in patients with systemic sclerosis was investigated using fluorescence microlymphography. The distal upper limbs of 16 healthy controls (mean age 62.3 ± 13.1 yr) and 16 patients with systemic sclerosis (mean age 58.9 ± 13.6 yr) were examined and the following parameters were evaluated: (a) single lymphatic capillaries; (b) lymphatic capillary network and cutaneous backflow; (c) extension of the stained lymphatics; (d) diameter of single lymphatic capillaries.

Results. At the finger level, lymphatic capillaries were lacking in five patients, while they were present in all controls ($P < 0.05$). Extension of the stained lymphatics was increased in 11 patients (8.1 ± 6.0 mm) compared to the 16 healthy controls (2.0 ± 1.2 mm) ($P < 0.0001$). Cutaneous backflow was observed in three patients ($P < 0.05$). At the hand level, lymphatic network extension was significantly different between patients (3.8 ± 2.4 mm) and controls (1.2 ± 0.8 mm) ($P < 0.01$); however, no significant differences were found at the forearm level.

Conclusion. Lesional skin in patients with systemic sclerosis exhibits evidence of lymphatic microangiopathy.

KEY WORDS: Systemic sclerosis, Lymphatics, Skin, Microangiopathy, Fluorescence microlymphography.

Patients with systemic sclerosis show cutaneous blood capillary microangiopathy characterized by dilated capillaries, avascular fields and increased capillary permeability [1, 2].

There are neither histological nor *in vivo* investigations dealing with the microlymphatic system of the skin in this disease. This is surprising because lymphatic microangiopathy plays an important role in other diseases with oedema, such as lymphoedema or chronic venous insufficiency [3–5], and oedema is an important part of the skin involvement in systemic sclerosis. To our knowledge, there are only two publications dealing with conventional lymphography in patients with systemic sclerosis [6, 7]. In a case report, rarefication of the peripheral lymphatic channels was described [6]. Rauste [7] published a series of 349 lymphographies in granulomatous inflammations and connective tissue diseases. He had incorporated two patients with scleroderma, of whom one had pathological lymphographic findings.

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The skin involvement of systemic sclerosis begins on the fingers and hands in most cases, and the changes usually proceed through three phases: early, classical and late. The oedematous early phase often begins with painless swelling of the fingers and dorsum of the hand. The subsequent classical indurative phase is characterized by firm, taut, hidebound skin. In the late atrophic phase, the skin of the hands shows the ravages of the early active fibrotic period. Other skin manifestations include digital pitting scars, loss of finger-pad tissue, ulcers, telangiectasia and calcinosis [8–11]. The clinical impression that the oedema of the early phase regresses in the later course of the disease is discussed controversially [11]. In fact, some data suggest that it does not. In one study, uniform and trimmed punch biopsy specimens from the forearm of patients with systemic sclerosis were weighed before and after desiccation. The percentage of tissue water weight was remarkably consistent in all patients (around 70%), irrespective of their clinical classification, duration of disease, or degree of clinical skin thickness and oedema at the site of biopsy [12]. Similar observations were noted in studies of skin core biopsy weights [13]. It is possible that the oedema does not resolve, but that the clinical ability to detect such changes becomes more limited as the dermis becomes more fibrosed [11].

In the literature, there are several hypotheses for the origin of the oedema in systemic sclerosis. It may be due to deposition of hydrophilic glycosaminoglycan in the dermis, local inflammation, hydrostatic effects or enhanced microvascular permeability [11]. These factors may in part account for the oedema. As an additional explanation, involvement of the cutaneous lymphatic system in the oedema development may be postulated.

The purpose of this study was, therefore, to investigate the microlymphatic system of the skin at the distal upper extremity in patients with systemic sclerosis.

Patients and methods

Healthy controls and patients

The group of 16 healthy controls comprised 12 women and four men (mean age $\pm$ S.D. 62.3 $\pm$ 13.1 yr; range 38–81 yr). None of them had actual finger swelling, previous history of swelling or clinical signs of connective tissue disease.

The 16 patients with systemic sclerosis comprised 14 women and two men (mean age $\pm$ S.D. 58. 9 $\pm$ 13.6 yr; range 33–76 yr). The patients are participating in a longitudinal assessment of the Swiss League against Rheumatism and had a work-up with extensive clinical examinations. If not previously performed, laboratory testing, chest X-ray, electrocardiography, echocardiography, respiratory function tests (spirometry, plethysmography and pulmonary diffusion capacity) and, if clinically indicated, cineradiography of the oesophagus, were conducted. All patients fulfilled the American College of Rheumatology (ACR) Scleroderma Cooperative Study Criteria [14] for the disease, i.e. one major criterion (proximal scleroderma) or two or more minor criteria (sclerodactyly, digital pitting scars of fingertips or loss of substance of the distal finger pad, bilateral basilar pulmonary fibrosis). Eleven patients had the limited and five patients the diffuse form of systemic sclerosis. The mean duration of the disease was 10.0 $\pm$ 13.3 yr (range 1–49 yr). Fifteen patients suffered from secondary Raynaud's phenomenon (94%) and 15 patients had sclerodactyly (94%). Actual or previous trophic changes to the fingertips were present in 15 patients (94%). Five patients had finger swelling at the time of investigation (31%). Telangiectasis of the skin was found in 14 patients (88%). Dysphagia or dyspepsia was present in 11 patients (69%) and cineradiography of the oesophagus was abnormal in six of 10 patients investigated. Pulmonary diffusion capacity was reduced in 10 patients (63%). Advanced renal insufficiency was not found among these patients. Two patients had mild renal insufficiency (creatinine level >105 μmol/l, but <125 μmol/l) (13%). Antinuclear antibodies were positive in 15 patients (94%), anti-Scl-70 antibodies in seven patients (44%) and anti-centromere antibodies in three patients (19%).

Technique of investigation

The investigation was based on the technique of fluorescence microlymphography, which has been described in detail previously [15, 16]. Large molecules are exclusively drained by the lymphatic system. Therefore, microlymphatics may be visualized by macromolecular, fluorescent materials injected into the subepidermal layer of the skin. From the original interstitial deposit, the dye moves into the superficial network of lymphatic microvessels and renders them visible. The anatomy of this system is well known from anatomical studies [17, 18].

Using a steel microcannula with a tip diameter of 0.2 mm (Arnold Bott, Zurich, Switzerland) connected to a microsyringe (Hamilton, Bonaduz, Switzerland), 10 μl of a sterilized 25% solution of FITC–Dextran (fluorescein isothiocyanate–dextran 150 000 molecular weight; Sigma Chemical, St Louis, MO, USA) were injected into the subepidermal layer of the skin. The injected FITC–Dextran was visualized by a fluorescence videomicroscopy system consisting of an incident-light fluorescence microscope with a mercury vapour lamp (Leica, Heerbrugg, Switzerland) mounted on a heavy support (Foba, Zurich, Switzerland), a 3-CCD videocamera (Model DXC-930, Sony, Tokyo, Japan) with a camera adapter (CMA-D2, Sony), a video timer and scale marker (For-A-Company, Tokyo, Japan), a videomonitor (Picture Monitor Model PM 171T, Ikegami Tsushinki, Tokyo, Japan) and a video recorder (S-VHS, AG-7350-E, Panasonic, Osaka, Japan). The microscope was equipped with 1.0/0.04, 2.5/0.08, 6.3/0.20 and 10/0.25 planar objectives (Leica, Heerbrugg, Switzerland), which allow a magnification of $\times 24$, $\times 62$, $\times 165$ and $\times 240$ on the monitor, respectively. The fluorescence excitation filter worked at 450–490 nm and the barrier filter at 515 nm.

The subjects were sitting on a chair and the hand was put on the stage of the microscope. The measurements were performed after a resting time of at least 10 min in a temperature-controlled room (22–24°C).

First, conventional microscopy of the nailfold blood capillaries of the third finger of the right hand was performed using the 2.5/0.08, 6.3/0.20 and 10/0.25 planar objectives (magnification $\times 62$, $\times 165$ and $\times 240$, respectively). Then, fluorescence microlymphography was performed on the dorsum of the middle phalanx of the middle finger of the right hand. After injection of 0.01 ml of dye into the subepidermal layer of the skin, the deposit and the filling of the lymphatic capillaries were observed during a time period of 10 min and stored on videotape. The planar objective 1.0/0.04 (magnification $\times 24$) was used during the whole observation time and after 10 min was changed to the 2.5/0.08 (magnification $\times 62$) objective. The whole observation field was then re-examined using this magnification. In the case of absence of visualized lymphatic capillaries, a second injection was performed to confirm the finding.

The same procedure was used at the dorsum of the hand in all subjects and at the dorsal proximal forearm in all healthy controls and in six patients (all clinically without involvement of the skin in this area).

The study protocol was approved by the ethical committee of the Department of Internal Medicine,

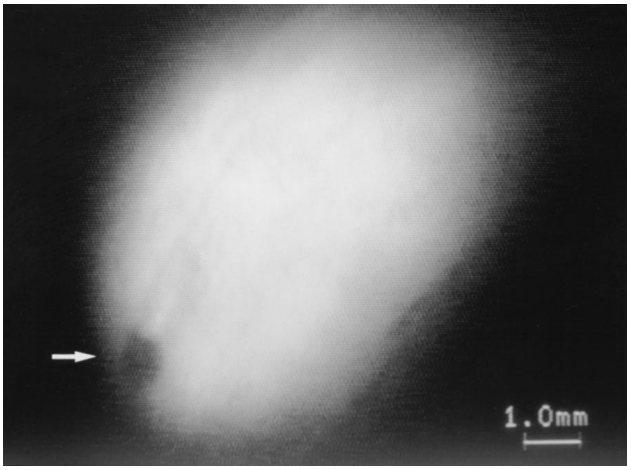

FIG. 1. Fluorescence microlymphography at the dorsum of the finger 10 min after injection of the dye in a 66-yr-old female patient with systemic sclerosis for 49 yr. Only the dye deposit is visible, no lymphatic capillaries are stained at all. Magnification ×24. Left, distal; above, ulnar. The arrow is the injection site.

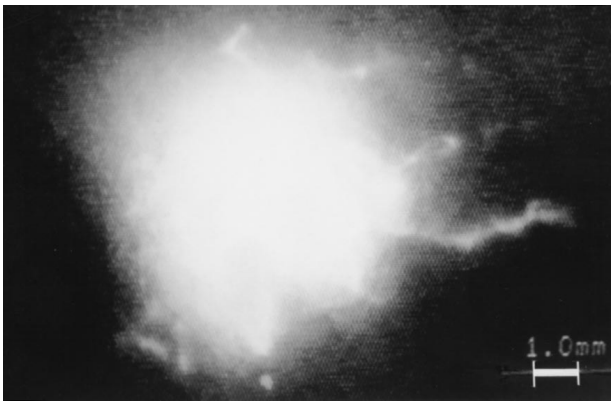

FIG. 2. Fluorescence microlymphography at the dorsal forearm 10 min after injection of the dye in a 50-yr-old female healthy control. Single lymphatic capillaries are visualized. Magnification ×24. Left, distal; above, ulnar.

University Hospital, Zurich. Healthy controls and patients gave written informed consent for the investigation.

Measurements

The following parameters were evaluated off-line from the videotape:

(a) Presence or absence of:
blood capillary microangiopathy (patients with avascular fields or giant blood capillaries (capillary diameter $>50\ \mu$m) were defined as having microangiopathy of the blood capillaries [1]);
visualized lymphatic capillaries 10 min after dye injection;
a superficial lymphatic capillary network (defined as at least three lymphatic capillary meshes) 10 min after dye injection;
fragmentation of the superficial lymphatic capillary network 10 min after dye injection (defined as lymphatic capillary network with interruptions of the meshes);
cutaneous backflow (reappearance of the fluorescent dye at the surface in islets composed of depicted meshes away from the main network) at 10 min.

(b) Maximal intralymphatic extension of the dye apart from the depot in the proximal, distal, radial and ulnar direction, as well as maximal extension 10 min after dye injection.

(c) Diameter of single lymphatic capillaries at 10 min (only subjects in whom at least three lymphatic

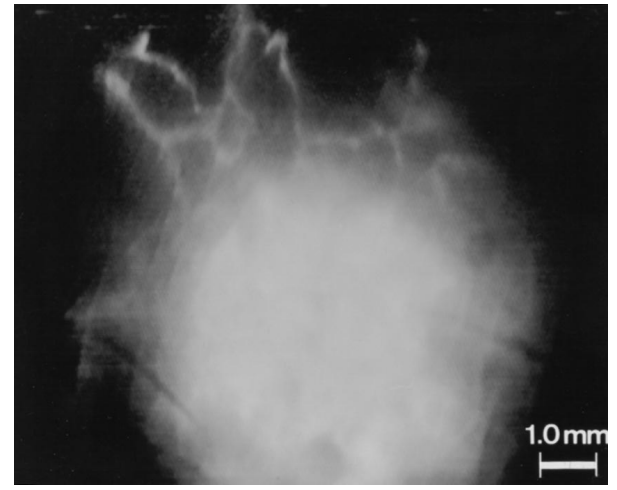

FIG. 3. Fluorescence microlymphography at the dorsum of the hand 10 min after injection of the dye in a 48-yr-old female healthy control. A lymphatic capillary network consisting of a few meshes is stained. Magnification ×62. Left, distal; above, ulnar.

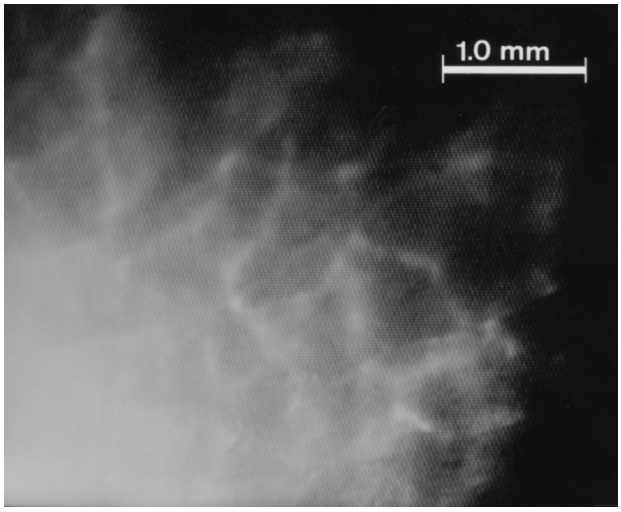

FIG. 4. Fluorescence microlymphography at the dorsum of the hand 10 min after injection of the dye in a 33-yr-old female patient with systemic sclerosis for 1 yr. A large lymphatic network is stained. Magnification ×62. Left, distal; above, ulnar.

capillaries could be measured were considered and the mean value of at least three measurements at different sites of the same capillary was taken).

Statistical analysis

Statistical analysis was performed on a personal computer (Macintosh II ci, Apple Computer, Cupertino, CA, USA) with use of a statistical program (Stat View 4.02, Abacus Concepts, Berkeley, CA, USA). Results are expressed as means $\pm$ S.D. χ^2 test or the Mann–Whitney *U*-test were used as appropriate. $P \leqslant 0.05$ was considered significant.

Results

Nailfold blood capillary microscopy

Blood capillary microangiopathy was present in none of the healthy controls, but in 12 of 14 patients. In two patients, capillary microscopy was inconclusive because of impaired skin transparency. Another two patients showed normal blood capillaries at the nailfold (one woman with the diffuse type of systemic sclerosis and a disease duration of 9 yr, and another female patient with the limited form and a disease duration of 49 yr). Of the 12 patients with blood capillary microangiopathy, 10 exhibited both giant capillaries and avascular fields. Two patients had avascular fields only.

Lymphatic capillary microscopy

Dorsum of the finger. Ten minutes after injection of the fluorescent dye, 13 controls showed single lymphatic capillaries (Table 1). In another three, a lymphatic capillary network was visible. By contrast, in five patients the absence of any lymphatic microvessel was observed ($P < 0.05$). Single lymphatic capillaries were visualized in five patients, and six patients exhibited a lymphatic capillary network. Fragmentation of the lymphatic capillary network was found in three of the six patients presenting a capillary network, but in none of the three corresponding controls [not significant (n.s.)] (Figs 1–4). Cutaneous backflow of the dye was exclusively observed in patients (3/16; $P < 0.05$ *vs* control). Mean maximal extension of the visualized lymphatic network was significantly increased in patients compared to controls (8.1 ± 6.0 *vs* 2.0 ± 1.2 mm; $P < 0.0001$), which also accounted for the extension of the network in the proximal, distal, ulnar and radial directions. No statistically significant difference between the groups was found for mean diameter of the lymphatic capillaries (Table 1). Table 2 gives the results of the finger investigation for the patients, subdivided into three disease duration groups, and although the numbers are small, helps to illustrate trends.

Dorsum of the hand. In all healthy controls, at least one lymphatic capillary was visible and three subjects showed a lymphatic capillary network. In contrast, there was a lack of lymphatics in three patients, whereas nine exhibited single capillaries and four showed a lymphatic capillary network. In parallel to the findings of the dorsum of the finger, a fragmented network was observed in two patients, but in none of the controls (n.s.). Again, maximal extension of the lymphatic network was significantly greater in patients compared to controls, involving also the proximal, distal, ulnar and radial directions. Mean diameters of the lymphatic capillaries were comparable in both groups (Table 1).

Forearm. As with the other sites investigated, all healthy controls exhibited at least one lymphatic capillary, extending to a capillary network in seven subjects. At the same time point, two of the six patients investigated had a lymphatic capillary network and four had single lymphatic capillaries (all patients without clinical scleroderma of the forearm; n.s.). No fragmentation or cutaneous backflow was observed in patients and controls. In addition, extension of the lymphatic network and the mean diameter of lymphatic capillaries were similar in both groups (Table 1).

Correlation between microlymphography and clinical findings

The correlation analysis revealed a significant positive correlation between duration of disease and lack of lymphatic capillaries at the finger ($P < 0.05$). No correlation could be found between the other parameters evaluated by fluorescence microlymphography or blood capillary microscopy and all other clinical data (i.e. gender, age, type of systemic sclerosis, Raynaud's phenomenon, finger swelling, sclerodactyly, trophic changes, telangiectasis, dysphagia or dyspepsia, pulmonary diffusion capacity, renal function, antibodies).

Discussion

These data represent the first intravital investigations to study the microlymphatic system of the skin in systemic sclerosis. The technique of fluorescence microlymphography [15] was used, which allows visualization and investigation of the superficial cutaneous lymphatic capillary network.

The main findings of this study were as follows: in clinically affected areas, patients with systemic sclerosis showed the pattern of lymphatic microangiopathy, known from patients with lymphoedema and chronic venous insufficiency [3–5], characterized by increased extension of the visualized lymphatic capillaries and cutaneous backflow or even the complete absence of stained microlymphatics. The frequency of these findings increased from the proximal to the distal hand. No significant differences were found at the forearm, which, however, was not clinically affected.

The finding of an increased extension of the fluorescent dye in the superficial lymphatic capillary network is known from patients with primary or secondary lymphoedema of both the upper or lower legs, and from patients with chronic venous insufficiency [5, 19–21]. It is the most important parameter of this technique for the diagnosis of lymphoedema and mild chronic venous insufficiency.

The phenomenon of cutaneous backflow (reappearance of the fluorescent dye at the surface in islets

TABLE 1. Fluorescence microlymphography at the dorsum of the finger, the dorsum of the hand and the dorsal forearm 10 min after injection of the dye FITC–Dextran: findings and measurements. Values are expressed as numbers of subjects or as means ± S.D.

	Finger			Hand			Forearm		
	Controls $n = 16$	Patients $n = 16$		Controls $n = 16$	Patients $n = 16$		Controls $n = 16$	Patients $n = 6$	
Single lymphatic capillaries	13	5		13	9		9	4	
Lymphatic capillary network	3	6		3	4		7	2	
No visible lymphatic capillaries	0	5	$P < 0.05$	0	3	n.s.	0	0	n.s.
Fragmentary network	0/3	3/6	n.s.	0/3	2/4	n.s.	0/7	0/2	n.s.
Cutaneous backflow	0/16	3/16	$P < 0.05$	0/16	0/16	n.s.	0/15	0/6	n.s.
Maximal network extension (mm)	2.0 ± 1.2	8.1 ± 6.0	$P < 0.0001$	1.8 ± 0.8	3.8 ± 2.4	$P < 0.01$	4.9 ± 1.8	8.8 ± 9.1	n.s.
Proximal extension (mm)	1.1 ± 1.0	7.1 ± 6.5	$P < 0.001$	1.2 ± 1.0	2.5 ± 1.3	$P < 0.05$	1.9 ± 1.6	4.0 ± 3.0	n.s.
Distal extension (mm)	0.8 ± 1.1	3.5 ± 3.0	$P < 0.01$	0.4 ± 0.5	1.7 ± 1.3	$P < 0.01$	2.4 ± 1.5	4.3 ± 6.0	n.s.
Ulnar extension (mm)	1.4 ± 0.8	3.0 ± 1.8	$P < 0.05$	0.9 ± 0.9	2.1 ± 1.5	$P < 0.05$	2.7 ± 1.8	4.8 ± 3.5	n.s.
Radial extension (mm)	0.6 ± 1.0	4.1 ± 2.3	$P < 0.001$	0.8 ± 0.9	2.5 ± 2.4	$P < 0.05$	3.2 ± 2.1	7.5 ± 9.8	n.s.
Lymphatic capillary diameter (mm)	0.133 ± 0.06	0.094 ± 0.02	n.s.	0.110 ± 0.03	0.094 ± 0.02	n.s.	0.119 ± 0.04	0.076 ± 0.01	n.s.

n.s., not significant.

TABLE 2. Fluorescence microlymphography at the dorsum of the finger. The patients are subdivided into three disease duration groups. Although the numbers are small, these data illustrate trends

	0–5 yr $n = 9$	P	6–15 yr $n = 5$	P	>15 yr $n = 2$
Single lymphatic capillaries	6		0		0
Lymphatic capillary network	3		3		0
No visible lymphatic capillaries	0	<0.05	2		2
Fragmentary network	2/3	n.s.	1/3		–
Cutaneous backflow	3/9	n.s.	0/5	n.s.	0/2
Maximal network extension (mm)	5.6 ± 2.1	<0.05	14.7 ± 8.6		–
Lymphatic capillary diameter (mm)	0.087 ± 0.09	n.s.	0.112 ± 0.02		–

composed of depicted meshes away from the main network) is another well-known finding in patients with lymphoedema or chronic venous insufficiency, not known in a healthy population [5, 20, 21].

It is assumed that increased dye expansion into the superficial lymphatic capillary network and cutaneous backflow are the result of an impeded drainage of interstitial fluid from the skin into the deeper main collectors. Thus, the superficial lymphatic capillary network acts as an overflow basin [22]. The underlying causes in these patients include aplasia, hypoplasia or, in patients with secondary lymphoedema, destruction of large lymphatic collectors [23].

Fragmentation of the lymphatic capillary network, i.e. damaged microlymphatics, is found in patients with lymphoedema following erysipelata [20], or in patients with severe chronic venous insufficiency [5, 24].

Complete destruction of the superficial lymphatic capillary network is known from patients with severe recurrent erysipelata or with severe chronic venous insufficiency [5, 20]. In these patients, several subepidermal dye injections fail to visualize any lymphatic microvessels. The same situation is also found in patients with Nonne–Milroy's disease, a congenital form of primary lymphoedema, where aplasia of the superficial lymphatic network of the skin takes place [25].

The finding that the severity of the lymphatic microangiopathy increases from proximal to distal at the hand correlates with the clinical finding that the skin alteration shows a similar pattern of involvement.

In chronic venous insufficiency, the general concept is that increased transcapillary diffusion of the blood capillaries leads to an increased amount of fluid and macromolecules in the interstitial space which initially is drained by compensatory augmentation of lymph flow. Lymphatic capillary morphology remains intact in early stages. With progression of the disease and the worsening of blood capillary microangiopathy, lymphatic microangiopathy contributes to oedema formation. Lymphatic involvement may be aggravated by recurrent infections. In lymphoedema, maximal augmentation of lymph flow occurs due to aplasia, hypoplasia or destruction of larger lymphatic vessels. Finally, the system becomes overloaded and insufficient, and the same mechanisms as in chronic venous insufficiency occur [3].

The patients of this study had a broad range of disease duration. It is, therefore, not surprising that there is also a vast range of pathology. However, there is a correlation of disease duration and complete disappearance of the lymphatic network, at least at the dorsum of the finger. This finding supports the hypothesis of a cascade of lymphatic microangiopathy in systemic sclerosis: leakage of the skin blood capillaries—increased amount of fluid and macromolecules in the interstitial space—maximal augmentation of lymph flow—finally lymphatic insufficiency—protein-rich interstitial fluid retention—fibrotic process and further damage to the microlymphatics. Thus, a vicious circle similar to chronic venous insufficiency may occur.

The finding of unchanged capillary diameters in patients with systemic sclerosis is not surprising as the same situation is also found in patients with lymphoedema [26].

In conclusion, lymphatic microangiopathy may, at least in part, explain oedema in patients with systemic sclerosis. The empirical finding that manual lymph drainage improves the swelling in these patients [27] seems to have a rational basis. It is possible that another classical treatment in patients with lymphoedema, compression therapy, may also have a beneficial effect, at least in the oedematous stage of the disease. The same can be postulated for drug treatment [28].

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References

1. Maricq HR, Harper FE, Khan MM, Tan EM, LeRoy EC. Microvascular abnormalities as possible predictors of disease subsets in Raynaud phenomenon and early connective tissue disease. Clin Exp Rheumatol 1983;1:195–205.
2. Bollinger A, Jäger K, Siegenthaler W. Microangiopathy of progressive systemic sclerosis, evaluation by dynamic fluorescence video microscopy. Arch Intern Med 1986;146:1541–5.

3. Bollinger A, Fagrell B. Clinical capillaroscopy, a guide to its use in clinical research and practice. Lewiston, NY: Hogrefe and Huber, 1990.
4. Leu AJ, Hoffmann U. Initial lymphatics of the skin: from basic research to clinical implications. J Vasc Invest 1997;3:143–8.
5. Leu AJ, Leu HJ, Franzeck UK, Bollinger A. Microvascular changes in chronic venous insufficiency—a review. Cardiovasc Surg 1995;3:237–45.
6. Röder K, Sieler H. Lymphographische Untersuchung bei progressiver Sklerodermie. Z Inn Med 1972;27:80–2.
7. Rauste J. Lymphographic findings in granulomatous inflammations and connective tissue disease. Acta Radiol 1972;suppl. 317:3–79.
8. Maddison PJ, Isenberg DA, Woo P, Glass DN. Oxford textbook of rheumatology. Oxford: Oxford University Press, 1993.
9. Black CM. Systemic sclerosis—management. In: Klippel JH, Dieppe PA, eds. Rheumatology, 2nd edn. London: Mosby, 1998.
10. Wigley FM. Systemic sclerosis—clinical features. In: Klippel JH, Dieppe PA, eds. Rheumatology, 2nd edn. London: Mosby, 1998.
11. Kelley WN, Harris ED, Ruddy S, Sledge CB. Textbook of rheumatology, 5th edn. Philadelphia: WB Saunders Co., 1997.
12. Furst DE, Seibold JR, Steen VD *et al*. The modified Rodnan skin core is an accurate reflection of skin thickness in systemic sclerosis. Arthritis Rheum 1995;38(suppl. 9):S334 (Abstract).
13. Rodnan GP, Lipinski E, Luksick J. Skin thickness and collagen content in progressive systemic sclerosis and localized scleroderma. Arthritis Rheum 1979;22:130–40.
14. Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581–90.
15. Bollinger A, Jäger K, Sgier F, Seglias J. Fluorescence microlymphography. Circulation 1981;64:1195–200.
16. Leu AJ, Berk DA, Yuan F, Jain RK. Flow velocity in lymph capillaries of the skin measured in a new model using the nude mouse tail. Am J Physiol 1994;267(Heart Circ Physiol 36):H1507–13.
17. Kubik S, Manestar M. Anatomy of the lymph capillaries and precollectors of the skin. In: Bollinger A, Partsch H, Wolfe JHN, eds. The initial lymphatics, new methods and findings. Stuttgart: Thieme, 1985.
18. Schmid-Schönbein GW. Microlymphatics and lymph flow. Physiol Rev 1990;70:987–1028.
19. Baer-Suryadinata C, Clodius L, Isenring G, Bollinger A. Lymph capillaries in postmastectomy lymphedema. In: Bollinger A, Partsch H, Wolfe JHN, eds. The initial lymphatics, new methods and findings. Stuttgart: Thieme, 1985.
20. Isenring G, Franzeck UK, Bollinger A. Fluoreszenz-Mikrolymphographie am medialen Malleolus bei Gesunden und Patienten mit primärem Lymphödem. Schweiz Med Wochenschr 1982;112:225–31.
21. Jäger K, Isenring G, Bollinger A. Fluorescence microlymphography in patients with lymphedema and chronic venous incompetence. Int Angiol 1983;2:129–36.
22. Bollinger A, Partsch H, Wolfe JHN (eds) The initial lymphatics, new methods and findings. Stuttgart: Thieme, 1985.
23. Kinmonth JB. The lymphatics, surgery, lymphography and diseases of the chyle and lymph systems. London: Arnold, 1982.
24. Bollinger A, Isenring G, Franzeck UK. Lymphatic microangiopathy: A complication of severe chronic venous insufficiency. Lymphology 1982;15:60–5.
25. Bollinger A, Isenring G, Franzeck UK, Brunner U. Aplasia of superficial lymphatic capillaries in hereditary and connatal lymphedema (Milroy's disease). Lymphology 1983;16:27–30.
26. Pfister G, Saesseli B, Hoffmann U, Geiger M, Bollinger A. Diameters of lymphatic capillaries in patients with different forms of primary lymphedema. Lymphology 1990;23:140–4.
27. Földi M, Casley-Smith JR. Lymphangiology. Stuttgart: Schattauer, 1983.
28. Casley-Smith JR, Morgan RG, Piller NB. Treatment of lymphedema of the arms and legs with 5,6-benzo-α-pyrone. N Engl J Med 1993;329:1158–63.