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Microangiopathy of Cutaneous Blood and Lymphatic Capillaries in Chronic Venous Insufficiency (CVI)*

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The severity of microangiopathy in patients with chronic venous insufficiency (CVI) determinates the extent of the trophic disturbances of the skin. Resulting from valvular incompetence of deep and/or perforating veins and the accompanying venous outflow obstruction caused by deep venous thrombosis (DVT), the increased ambulatory venous pressure heads are transmitted retrograde into the microvasculature of the skin at the ankle region. In the present study, we have assessed the changes in the cutaneous microvasculature by dynamic fluorescence video microscopy, fluorescence microlymphography, and transcutaneous oxygen tension ($tcPO_2$) measurements.

In mild forms of CVI, capillary density, morphologic characteristics, and $tcPO_2$ are still normal. Fluorescent light intensity is, however, significantly increased, indicating an increased transcapillary diffusion of sodium fluorescein (NaF) as a marker for enhanced leakage of the capillaries in the early stage of the disease. The pericapillary halo diameters are significantly enlarged, compared to controls ($p < 0.01$).

In the severe stages of CVI and in patients with venous ulcers, capillary thromboses, probably caused by endothelium-blood cell interactions, may lead to a reduced capillary density. In order to enlarge the exchange surface area, the remaining skin capillaries become tortuous (capillary tufts). Parallel to the reduced capillary number, $tcPO_2$ decreases and can be extremely low at the ulcer rim or at white atrophy spots. Fibrin cuffs are not a specific finding for venous ulceration and do not significantly impair oxygen diffusion.

Fluorescence microlymphography permits visualization of the lymphatic capillaries of the superficial skin. In severe stages of CVI, the lymphatic capillary network at the medial ankle area is destroyed, and the remaining lymphatic capillary fragments have an increased permeability to FITC-dextran with a molecular weight of 150,000. These findings demonstrate a special lymphatic microangiopathy in CVI, suggesting an additional lymphatic component in the edema formation.

INTRODUCTION

Chronic venous insufficiency (CVI) is mainly caused by chronic obstruction and/or valvular insufficiency of the deep veins secondary to deep venous thrombosis (DVT) or by incompetent perforating veins. It is confined almost exclusively to the lower extremities and leads eventually to trophical skin changes and to venous ulceration at the medial ankle region. During walking, the pressure heads of ambulatory venous hypertension are transmitted retrograde via insufficient perforating veins to the skin

Abbreviations: CVI: chronic venous insufficiency DVT: deep venous thrombosis FITC: fluorescein-isothiocyanate FLI: fluorescent light intensities NaF: sodium fluorescein PO_2: partial oxygen pressure $tcPO_2$: transcutaneous oxygen tension

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microvasculature. This process leads to alterations of the nutritional capillaries and microlymphatics of the skin and also of the interstitial tissue.

Today new methods permit study of the arising morphologic and functional changes of the cutaneous microvasculature. In the following study, fluorescence video microscopy, transcutaneous oxygen tension measurements, and fluorescence microlymphography were used to assess these changes in patients with chronic venous insufficiency.

METHODS

Transcutaneous Oxygen Tension Measurements

Transcutaneous oxygen tension ($tcPO_2$) measurements have been described in detail before [1,2]. This method is basically a polarographic determination of molecular oxygen diffusing to the skin surface, by means of a modified Clark-type oxygen electrode. The partial oxygen pressure (PO_2) signal results from the reduction current at the cathode of the electrode, which is proportional to the amount of oxygen diffusing from the capillaries to the skin surface. This technique gives a quantitative measure for skin oxygenation.

A special probe, used in this study, permits the simultaneous observation of the capillaries at the oxygen sensing site by video microscopy [3]. The electrode consists of a single platinum cathode (tip diameter, 15 μm) melted in a glass cylinder and a silver-silver/chloride anode ring surrounding the transparent center portion.

A core temperature of 45°C, which results in an effective skin surface temperature of 43°C, was used to obtain maximal local flow conditions. Figure 1 shows the investigational set-up.

Fluorescence Video Microscopy

Dynamic fluorescence video microscopy [4] includes capillaroscopy of the skin by a video microscopy system before and after intravenous injection of the fluorescent dye sodium fluorescein (NaF). This fluorochrome is used to study transcapillary diffusion and to visualize structures, for example, the pericapillary space which cannot be depicted otherwise. Video microscopy is the only method that promotes direct information about the cutaneous microcirculation.

The apparatus consits of an incident light fluorescence microscope (Leica AG, Herbrugg, Switzerland) with a mercury vapor lamp (HBO 100W, Osram, Germany), a cadmium-selenide camera (c-vidicon, K30, Siemens, Karlsruhe, Germany), a video timer (For-A-Company, Chiba, Japan), a video scale marker (For-A-Company), a video monitor (Philipps, Eindhoven, The Netherlands), and a video tape recorder (S-VHS, Panasonic, Japan). The microscope and the camera are mounted on a special support (Leica AG, Glattbrugg, Switzerland), which permits optimal adjustment of the microscope to the skin [5].

Fluorescence Microlymphography

Unlike the blood capillaries of the skin, the cutaneous microlymphatics cannot be visualized without contrast media. Therefore a subepidermal injection of 0.01 ml FITC (fluorescein-isothiocyanate)-dextran with a molecular weight of 150,000 in a 20 percent solution is administered at the medial ankle region by means of a steel microcannula with an outer diameter of 0.2 mm. From an initial deposit of the

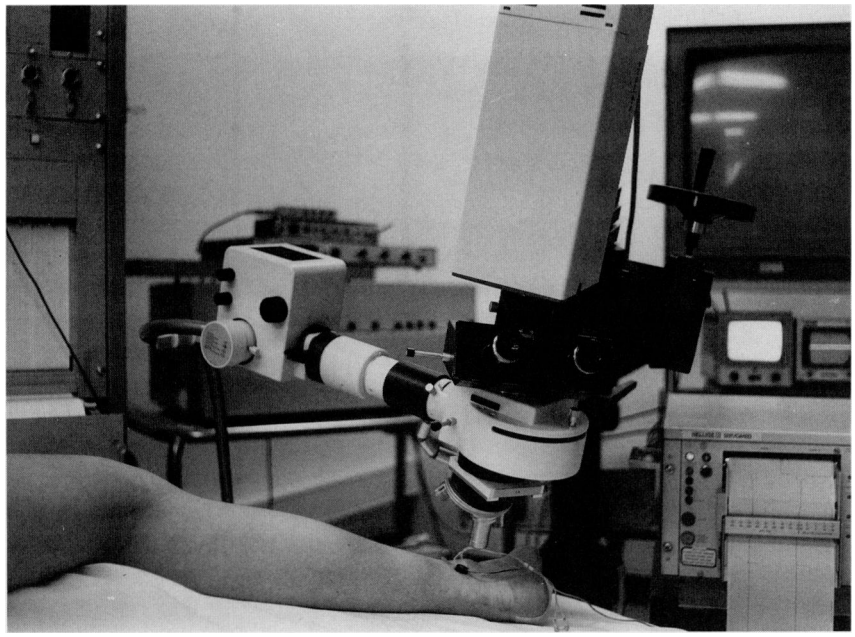

FIG. 1. Investigational set-up for the combined $tcPO_2$ measurements and video microscopy. The transparent oxygen probe is attached at the medial ankle region. The fluorescence light microscope is adjusted at the sensing site. The video camera is mounted, together with the microscope, on a solid stage which can be moved in any direction. On the right side, the oxymonitor for the measurements of transcutaneous oxygen tension can be seen and, in the background, the video monitor, the video tape recorder, and control instruments.

fluorescent dye, the lymphatic capillaries are stained. The lymphatic capillaries form a network, which is recorded by fluorescence video microscopy on video tape [6].

PATIENTS

Combined Transcutaneous PO_2—Capillary Morphology Study

Healthy Subjects: The group of 24 healthy subjects consisted of seven women and 17 men with a mean age of 48 years (range, 19–75 years). They had no clinical evidence of peripheral atherosclerotic or venous disease. In this group, 35 measurements of $tcPO_2$ were obtained (11 bilateral measurements).

Patients: The CVI group included 17 patients with a mean age of 56 years (range, 22–76 years). Forty-four measurements were obtained in this group. In 12 patients, CVI was caused by deep venous thrombosis, and five patients suffered from primary varicose veins with incompetent perforating veins. Nine patients had trophic skin changes with ulcers or healed ulcers. The diagnosis was based on the patient's history, the clinical findings, and the results of Doppler ultrasound examination. Venograms were obtained in seven patients.

Fluorescence Video Microscopy—NaF Studies

Healthy Subjects: Fifteen healthy probands without evidence of CVI served as controls (mean age, 61 years; range, 35–73 years). Only volunteers with normal venous Doppler examinations were included.

Patients with Mild CVI: In this study group, 15 patients with lower limb edema, corona phlebectatica paraplantaris, and slight trophic skin changes were included. The mean age was 54 years (range, 28–75 years). Four patients had primary varicose veins (incompetence of perforating veins), and 11 patients exhibited post-thrombotic changes.

Patients with Severe CVI: The study group consisted of 15 patients with severe chronic venous insufficiency, with pronounced trophic skin changes, hyperpigmentation, and induration of the gaiter area. Ten patients had healed venous ulcers (mean age, 57 years; range, 47–73 years). Venous Doppler studies were performed in all patients. In seven patients, the findings were typical for obstruction and/or valvular insufficiency of deep veins (post-thrombotic syndrome), whereas, in eight patients, primary varicose veins with incompetent perforating veins were present.

Fluorescence Microlymphography Study

Healthy Subjects: In the control group, 15 healthy volunteers were included. Their mean age was 31 years (range, 23–45 years). A total of 17 microlymphographies were performed (twice bilateral).

Patients: Twenty-one patients (14 women, seven men) with a mean age of 51 years (range, 19–80 years) were in the CVI group. Thirty-two studies were performed (11 times bilateral examinations).

In 20 legs, CVI had developed as a consequence of deep venous thrombosis. In the remaining 12 legs, primary varicose veins with insufficient perforators were diagnosed.

RESULTS

Combined Transcutaneous PO_2—Capillary Morphology Study

Control Group: In 24 healthy subjects, mean $tcPO_2$ of the medial ankle region was 56.8 ± 9.9 mm Hg (range, 38–79 mm Hg).

The superficial skin capillaries were homogeneously distributed. The capillary density averaged 45 capillaries per mm^2. Depending on the skin transparency, usually only the apex of the capillary loops could be observed, because the skin capillary loops at the ankle area run perpendicular to the skin surface.

Patients with CVI: In CVI patients, the mean $tcPO_2$ was 47.7 ± 14.5 mm Hg (range, 21–83 mm Hg) when the measurements were performed at the medial ankle area above incompetent perforating veins and *without major trophic skin changes* ($p > 0.05$).

The simultaneous video microscopic examination at the oxygen sensing site revealed dilated and tortuous capillaries, which frequently appeared to be surrounded by a distinct halo structure. The number of capillaries was not significantly reduced ($p > 0.05$) at these sites (see Fig. 2).

Over *skin areas with moderate to severe trophic changes,* $tcPO_2$ averaged 22.5 ± 7.0 mm Hg (range, 13–26 mm Hg). This value is significantly lower ($p < 0.001$) than that in the control group and in the CVI group without trophic changes of the skin.

The capillary density was less than ten capillaries per mm^2 in these areas. The tortuosity and dilatation of the capillaries increased in proportion to the reduction in capillary number. The remaining capillaries that were observed were extremely dilated, with irregularly shaped arteriolar and venular limbs (capillary tufts). Capil-

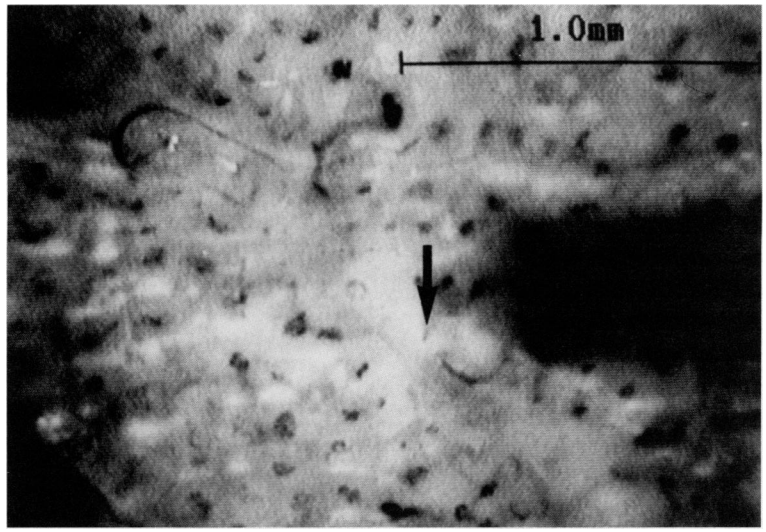

FIG. 2. View through the transparent oxygen electrode of normal capillary density and homogeneous distribution of capillaries in a patient with chronic venous incompetence without trophic changes. The arrow indicates the tip position of the platinum cathode, which is the oxygen sensor. Transcutaneous PO_2 was in the normal range and measured 60 mm Hg.

laries were counted after the study on single frames at the video monitor during playback of the video tape.

In the *center of white atrophy spots,* no capillaries were visible (avascular fields), whereas, in the border zones, they were enlarged and meandering. The capillaries sometimes had a glomerulus-like appearance, and mean $tcPO_2$ reached 24.0 mm Hg (range, 12–44 mm Hg) in these border zones. An example is shown in Fig. 3.

The large range of single PO_2 determinations is the result of the varying distances between oxygen sensor and adjacent capillaries in the border zone of white atrophy and in areas of decreased capillary density. This distance is of great importance for $tcPO_2$ measurements, since greater distance results in lower PO_2 values, and vice

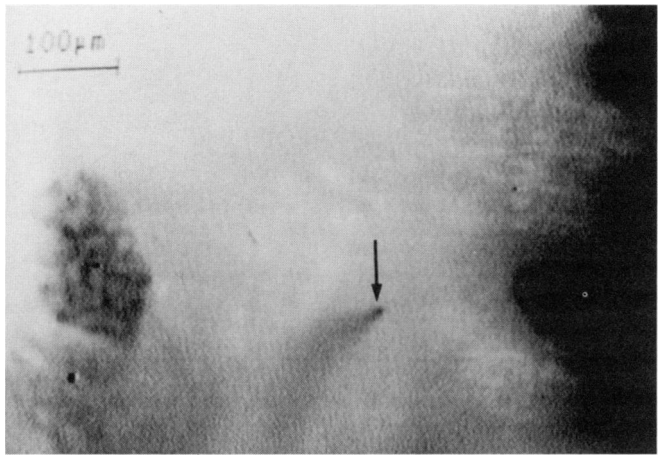

FIG. 3. View through the transparent oxygen electrode, which is attached on the center of a healed venous ulcer. The arrow in the middle indicates the position of the oxygen sensor (platinum cathode of the electrode). At the right and left sides, tortuous capillaries can be seen. The white areas are avascular fields. The $tcPO_2$ was extremely reduced at this measuring site (0 mm Hg).

TABLE 1
Fluorescent Light Intensities (FLI) in Healthy Subjects and Patients with Mild and Severe Chronic Venous Insufficiency (CVI)

Time	Controls (n = 15) FLI (%)	p-value[a]	Patients with Mild CVI (n = 15) FLI (%)	p-value[b]	Patients with Severe CVI (n = 15) FLI (%)
1 second	1.4 ± 1.6	0.01	5.7 ± 5.2	0.001	0.7 ± 1.1
3 seconds	2.2 ± 1.9	0.01	7.8 ± 6.7	0.001	2.0 ± 2.1
5 seconds	3.6 ± 2.9	0.01	9.7 ± 8.1	0.01	3.7 ± 3.5
10 seconds	5.9 ± 5.0	0.05	13.0 ± 9.7	NS	10.2 ± 12.3
30 seconds	14.7 ± 9.0	0.05	24.1 ± 13.1	NS	20.6 ± 16.9
60 seconds	23.6 ± 11.1	NS	31.4 ± 12.6	NS	26.8 ± 14.9
2 minutes	36.9 ± 13.8	0.05	44.6 ± 9.9	NS	39.3 ± 13.1
5 minutes	62.6 ± 13.8	0.05	73.9 ± 10.4	NS	67.6 ± 11.5
10 minutes	86.6 ± 10.9	0.05	94.5 ± 6.0	0.05	89.8 ± 5.7
15 minutes	95.5 ± 5.2	NS	97.0 ± 3.5	NS	97.6 ± 4.3
20 minutes	96.8 ± 4.4	NS	91.5 ± 9.5	NS	93.4 ± 7.9

The measurements were performed using large-window video densitometry. Fluorescent light intensities (FLI) are given as mean values and standard deviations at different times after first appearance of sodium fluorescein in the skin capillaries (percentage of the maximum individual intensity).

[a]Comparison between controls and patients with mild CVI

[b]Comparison between patients with mild CVI and severe CVI; there were no significant differences between controls and patients with severe CVI (unpaired Wilcoxon test).

versa. The same situation applies to the center areas of white atrophy where no capillaries were observed. The $tcPO_2$ values were measured at 0 mm Hg or close to 0 mm Hg at these sites (see Fig. 3). The distance between the cathode tip of the oxygen sensor and the capillary was more than 100 μm when the $tcPO_2$ was extremely reduced.

Fluorescence Video Microscopy—NaF Studies

Healthy Subjects and Patients with Mild CVI: The appearance of the fluorescent dye in the capillaries of skin areas without trophic lesions was homogeneous in both groups. The transcapillary diffusion of NaF was significantly enhanced in patients, as indicated by the mean fluorescent light intensities, which are significantly increased ($p < 0.01$) until 15 minutes after dye appearance (Table 1). The maximum fluorescent light intensity (FLI) is reached earlier than in healthy controls ($p < 0.05$).

The mean diameter of the pericapillary space (halo) was significantly enlarged ($p < 0.001$), which is the expression of an initial pericapillary microedema. Whereas in healthy probands the mean diameter was 81 ± 15 μm, in patients with mild CVI the diameter amounts to 138 ± 13 μm. The distribution of single values in the control group is normal (Gaussian distribution). This distribution was not the case, however, in patients with CVI [7], which indicates that the enhanced capillary leakage is not a regular phenomenon but is inhomogeneously distributed.

Severe CVI: In contrast to healthy subjects, in whom all capillaries of the observation field were perfused within 32 seconds after the initial appearance of the dye (filling time), the appearance of NaF in the skin capillaries of patients with severe CVI was mainly asynchronous. The filling time was prolonged, and it could

TABLE 2
Extension of FITC-Dextran 150,000 in the Superficial Lymphatic Capillary Network in Healthy Subjects (n = 17 Studies) and Patients with CVI (n = 32 Studies)

Extension	Controls	Patients with CVI	Level of Significance
Proximal	3.5 ± 2.5	16.9 ± 24.4	$p < 0.05$
Distal	2.9 ± 2.1	9.3 ± 9.4	$p < 0.01$
Ventral	3.9 ± 2.9	14.8 ± 9.4	$p < 0.001$
Dorsal	7.1 ± 3.2	17.4 ± 9.6	$p < 0.001$

Mean values and standard deviations are given in mm.

last 64 seconds, until all capillaries of the observation field were perfused. These findings speak in favor of an increased heterogeneity of the microvascular perfusion in these severe stages.

In some patients, there can be found capillary convolutes filled with red blood cells and flow-stop, which are not perfused with the fluorescent dye even after 30 minutes. This finding is interpreted as *capillary thrombosis (microthrombosis).*

Whereas the transcapillary diffusion was no longer increased ($p > 0.05$) in severe CVI, compared to the control group [7] as an indicator of end-stage disease, the enlargement of the halo diameters was significant ($p < 0.001$).

Fluorescence Microlymphography Study

Mild CVI: In cases with CVI without trophic changes, no changes of lymphatic capillary morphology were observed. The propagation of the fluorescent dye was, however, significantly enhanced (refer to Table 2). The enlarged extension of the microlymphatic network is probably due to the increased lymphatic drainage caused by the enhanced leakage of the blood capillaries.

Moderate and Severe CVI: In patients with trophic changes in the medial ankle region, the superficial network of the lymphatic capillaries was damaged. The meshes which form a regular network in healthy controls and in patients without trophic skin changes were interrupted, only partially filled, or completely obliterated (Fig. 4). Fragments of lymphatic capillaries may be filled relatively far away from the deposit. In cases with no filling of capillaries at all, the dye moved diffusely into the interstitial space.

DISCUSSION

The present studies demonstrate a pronounced microangiopathy of blood and lymphatic capillaries in patients with severe chronic venous insuffiency as a consequence of the increased venous pressure during walking; this condition results mainly from valvular incompetence of the deep and perforating veins. In addition, venous outflow obstruction caused by deep venous thrombosis may contribute to the peripheral venous hypertension.

In the *initial stages of CVI,* the microangiopathy is characterized by dilatation of the blood capillaries. As a result of the proposed retrograde transmitted pressure heads,

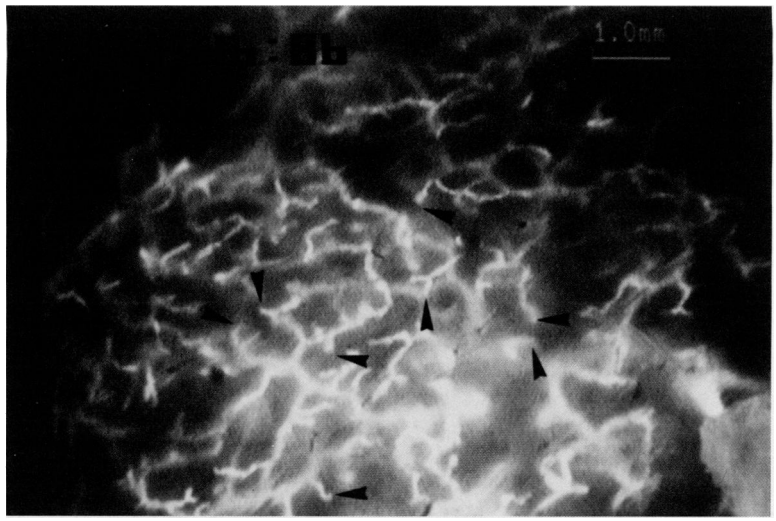

FIG. 4. Fluorescence microlymphographic image in a patient with severe chronic venous insufficiency. The measurement site is the medial ankle region. Note the large extension of the microlymphatic network and especially the obliteration of single lymphatic capillaries (*arrows*). Part of the initial deposit of the fluorochrome FITC-dextran can be seen in the lower right corner.

the capillaries eventually become dilated, tortuous, and form capillary convolutes or tufts [8,9]. So far, however, capillary pressure measurements in patients with chronic venous incompetence have not been performed because of technical difficulties; therefore it must be stated that the proposed mechanism is hypothetical and not yet proven.

The transcapillary diffusion of the low molecular fluorochrome sodium fluorescein is increased as a result of enhanced capillary leakage. The pericapillary space is enlarged, due to the developing microedema, and its diameters are no longer normally distributed but show a wider range than that in healthy subjects.

The morphology of lymphatic capillaries is still normal in the initial disease. The extension of the FITC-stained network is already significantly increased, however, compared with control subjects [10]. This condition is caused mainly by the increased capillary leakage, which consequently leads to an increase in lymph fluid.

Transcutaneous oxygen tension is normal in this early stage of the disease, where capillary density at the sensing site is normal [9], which demonstrates a sufficient oxygen supply to the skin in the mild form of chronic venous incompetence. The normal range of transcutaneous oxygen tension is between 50 and 80 mm Hg.

In more *severe stages of CVI,* microthrombosis of capillaries can be found [11] contributing to the locally reduced capillary density at potential ulcer sites of the medial ankle region. Most likely these microthromboses are the consequence of interactions between leucocytes and other blood cells with the vessel wall of post-capillary venules. The capillaries are stuffed with red blood cell aggregates, which would not be the case if thrombosis were to start in the terminal arterioles or at the arterial side of the capillary loop. In such cases, the capillaries are void of corpuscular blood elements.

Transcutaneous oxygen tension decreases parallel to the reduction in capillary

number. The role of pericapillary fibrin cuffs as an oxygen diffusion barrier, indicated by the decreased PO_2 values, is unlikely. It was shown by Michel [12] that pericapillary cuffs do not impair oxygen diffusion. Vanscheidt et al. [13] found no clear relationship between the presence of fibrin cuff and $tcPO_2$; however, capillary density is the number one factor for reduced $tcPO_2$ values in CVI [9].

At the border zones of white atrophy spots, which are potential sites for venous ulcer formation, the capillary density is significantly reduced, and the capillaries are even more tortuous than in the mild stages of the disease. In these cases, the increase in capillary size is most likely necessary to increase the exchange area between intra- and extravascular compartments for fluids and soluble substances. Similar changes in capillary morphology are observed at cutaneous scar sites after injuries. The measurement of transcutaneous PO_2 depends on the distance between oxygen sensor and adjacent capillaries at such sites with reduced capillary number and irregular distribution of capillaries. The reduced PO_2 implies localized, spot-like ischemia of these areas, and additional minor external and internal factors may lead to the tissue damage and ulcer formation.

In the center of white atrophy, avascular fields are present, which result in enlarged diffusion distances and in significantly prolonged diffusion times [14]. In this stage of the disease, lymphatic microangiopathy progresses, and the lymphatic capillary network becomes destroyed and obliterated [10]. The diffusion of FITC-dextran 150,000 is enhanced. In severe cases, only fragments of lymphatic capillary can be found. These changes demonstrate an additional lymphatic component of edema formation in CVI.

Finally, venous ulceration is obviously caused by localized microvascular ischemia, which does not result only from one, but from multiple factors [15]. Further research is necessary in order to gain more direct evidence of the importance of white cells in the cascade of events leading to ulcer formation. We will also try to measure capillary pressures at the ankle area in those patients with impeding venous ulceration in order to prove the proposed mechanism of retrograde transmitted pressure elevation.

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