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Clinical Histochemical and Immunohistochemical Investigation of the Capillary Basal Membrane in Chronic Venous Insufficiency

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Present investigations regarding the pathogenesis of chronic venous insufficiency (CVI) are focusing on microvascular changes. The aim of our investigation was to examine the correlation between the thickness of pericapillary type IV collagen depositions, basal membrane alterations and transcutaneous oxygen tension ($TcPO_2$) in CVI-patients. Histochemical and immunohistochemical investigation of the capillary basal membrane was performed on 15 biopsies from normal controls, as well as 30 patients with CVI stage I and III (classification by Widmer & Stähelin). In all subjects $TcPO_2$ was measured just prior to biopsy procedures in exactly the same area where the specimen was subsequently excised. The microscopically measured thickness of the collagen IV layer and the basal membrane was increased significantly in patients with CVI. Specimens from normal controls showed a collagen IV layer thinner than 0.1 µm. Patients with CVI stage III revealed strong collagen IV depositions between 0.2 and 0.3 µm. Comparison between $TcPO_2$ and histological findings in the measured areas showed oxygen pressure varying from 62 mmHg (SEM 4.94 mmHg) in normal controls, down to 13 mmHg (SEM 3.39 mmHg) in patients with thick collagen IV layers. Besides an increased collagen IV layer, microvascular thrombosis and a thickened basal membrane have to be considered for impaired capillary perfusion. ***Key words: microcirculation; collagen IV; transcutaneous oxygen tension.***

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Until recently, research regarding chronic venous insufficiency (CVI) was based on the hemodynamic changes underlying this common disorder. At present, investigations are focusing on microvascular changes (1, 2). Investigations with methods like capillary microscopy, transcutaneous oxygen tension ($TcPO_2$) and new histochemical and immunohistochemical studies have raised controversial discussions.

In CVI a pericapillary halo has been demonstrated with different methods such as fluorescence-video-microscopy and capillary microscopy (3). The composition and pathogenic importance of these depositions around capillaries have been discussed extensively (4). Burnand et al. postulated that pericapillary fibrin depositions are the cause of venous ulceration (5–7). However, Vanscheidt et al. showed that pericapillary fibrin cuffs are associated with $TcPO_2$ tensions of up to 33 mmHg, demonstrating that pericapillary fibrin cuffs are not a sufficient diffusion barrier (8).

Neumann & van den Broek demonstrated a substantial increase of a type IV collagen layer in the basal membrane area (9). Having applied immunohistochemical staining of type IV collagen, Leu reported that patients with CVI have a multilayered, split-up capillary basal membrane with an ill-defined, frayed outer surface (10). To our knowledge, there have been no reports concerning a connection between the capillary type IV collagen cuff and $TcPO_2$ decrease in patients with severe CVI.

In this study, we combined clinical, histochemical and immunohistochemical methods to examine changes of the basal membrane in patients suffering from different stages of CVI and the significance of these changes for $TcPO_2$ decrease.

PATIENTS AND METHODS

Patients

The clinical and histochemical investigations included 15 healthy volunteers (control group), 15 patients with CVI stage I, and 15 patients with CVI stage III (classification by Widmer & Stähelin) (11). Patients with CVI stage I had been suffering from CVI for at least 6 months. In contrast, CVI stage III patients had been affected for more than 12 months. The mean age of the patients was 48 years (ranging between 25–76 years, 31 females and 14 males). Diagnosis was confirmed by history, Doppler sonography and phlebodynamometry. Patients with diabetic microangiopathy, arterial disorders and connective tissue diseases were excluded from this study. Informed written consent was obtained from all patients. After application of local anaesthesia (Scandicain 1%), 4-mm punch biopsies were taken 10 cm above the medial malleolus.

From patients with CVI stage I and III, biopsies were either taken from skin with obvious pathological alterations or from ulcer walls. During the same session, biopsies were obtained from undiseased skin medial below the knee.

All biopsies were fixed immediately in 4% formaldehyde (Riedel-de Haen AG, Seelze, Germany) and subsequently embedded in paraffin wax (Vogel Histo-Comp®, Giessen, Germany).

Histochemistry and immunohistochemistry

Serial cryostat sections (3 µm) were prepared using a Cryocut 2000 (Reichert & Jung, Nußbach, Germany). Sections were stained with hematoxylin-eosin (HE) and PAS stain. Serial sections were additionally stained with a monoclonal antibody against collagen IV (DAKO-Anti-Collagen IV, CIV 22, Dakopatts, Denmark) using the alkaline phosphatase anti-alkaline phosphatase technique (APAAP), as described previously (12).

Histological evaluation was performed in a double-blind fashion by two independent observers who were not aware of the $TcPO_2$ data, using a Zeiss Axioskop, equipped with a MC 100 camera system (Zeiss, Obercochem, Germany).

Evaluation of slides

In each specimen the epidermis, dermal capillaries, capillary basal membrane, capillary loops and pericapillary infiltrate were evaluated for pathological changes.

Thickness of pericapillary type IV collagen layer was examined and compared to basal membrane alterations after PAS-staining and $TcPO_2$ measurement. Thickness was evaluated microscopically at 10X magnification. Stages of pericapillary collagen IV layer thickness were

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classified as follows: 1) thin collagen IV layer (<0.1 μm); 2) medium thick collagen IV layer (0.1–0.2 μm) and 3) thick collagen IV layer (>0.2 μm). Evaluation of basal membrane-thickness after PAS-staining was performed identically.

Transcutaneous oxygen tension

To allow a correlation between the morphological changes of microvessels and functional alterations, $TcPO_2$ was measured just prior to biopsy procedures in exactly the same area where the specimen was subsequently excised.

$TcPO_2$ was measured on the supine patient after a 30-min adaption period at room temperature (22–24°C). $TcPO_2$ was measured for 1 h, always in the same room, the $TcPO_2$ electrode was adjusted to 44°C and constantly heated to this temperature.

RESULTS

Histological findings

Patients with CVI present with acanthosis, capillary dilatation, pericapillary infiltrate and microthrombi. In the group of healthy volunteers no pathological changes of the dermal capillaries of the stratum papillare or of the capillary basal membrane were visible. There was neither a pericapillary infiltrate nor microvascular thrombi. Similar results were found in control sections obtained just below the knee from CVI-patients stage I and III.

However, acanthotic changes of the epidermis were observed in 14 cases of CVI-patients, 4 stage I and 10 stage III. All sections in CVI stage III showed glomerulum-like distortion of capillaries and an increase of capillary loops in the upper corium. In CVI stage I capillary dilation was found in 9 specimens. Characteristic of CVI stage III was a lymphohistiocytotic infiltrate surrounding capillaries. Examination revealed microthrombi in 12 sections from CVI stage III patients, compared to only two sections in CVI stage I. Most of the microthrombi were located in the dermal capillaries of the stratum papillare.

Patients with CVI stage III have a thick multilayered basal membrane. In sections from the control group the capillary basal membrane could be detected as a thin, easily distinguishable band using PAS-staining (Fig. 1). A thin basal membrane was found in 6 patients with CVI stage I, and a medium thick basal membrane in 7 patients with lipodermatosclerosis. However, in 11 of 15 specimen, a thick multilayered basal membrane correlated strongly with CVI stage III (Fig. 2). The remaining patients showed medium thick basal membranes.

Patients with CVI stage III present with collagen IV depositions around capillaries. Evaluation of collagen IV depositions after immunohistochemical staining revealed thin collagen IV depositions in sections of the control group (Figs. 3, 5). In CVI I collagen IV layers thinner than 0.1 μm were found in 2 and collagen IV depositions thicker than 0.2 μm in 3 cases. Nine CVI I patients revealed medium thick collagen IV layers. In contrast, patients with CVI stage III revealed thick collagen IV depositions in 8 cases (Fig. 4). Medium thick and thin collagen layers were detected three times and twice, respectively (Fig. 5).

When comparing the thickness of the basal membrane after PAS-staining and pericapillary collagen IV deposition in the different groups, we found an almost identical distribution (Figs. 3, 4).

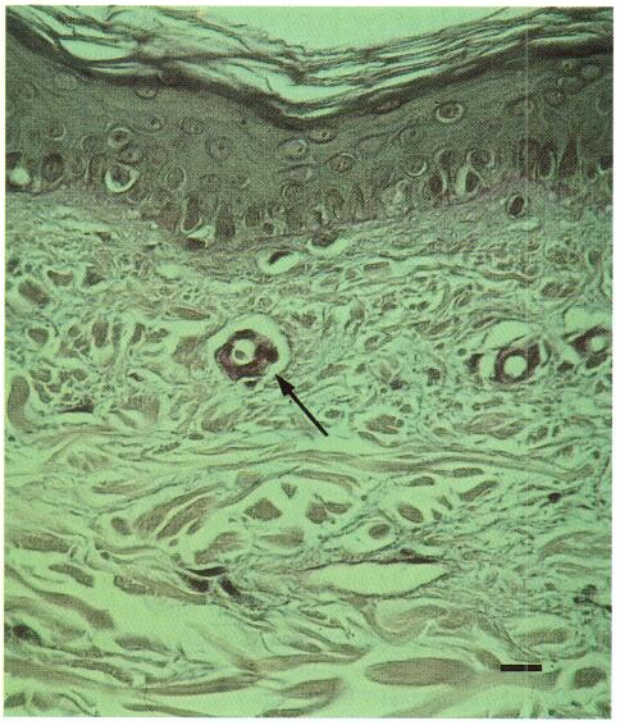

Fig. 1. Thin pericapillary basal membrane (→), easily distinguishable from surrounding tissue in normal skin (PAS-stain; *bar* = 16 μm).

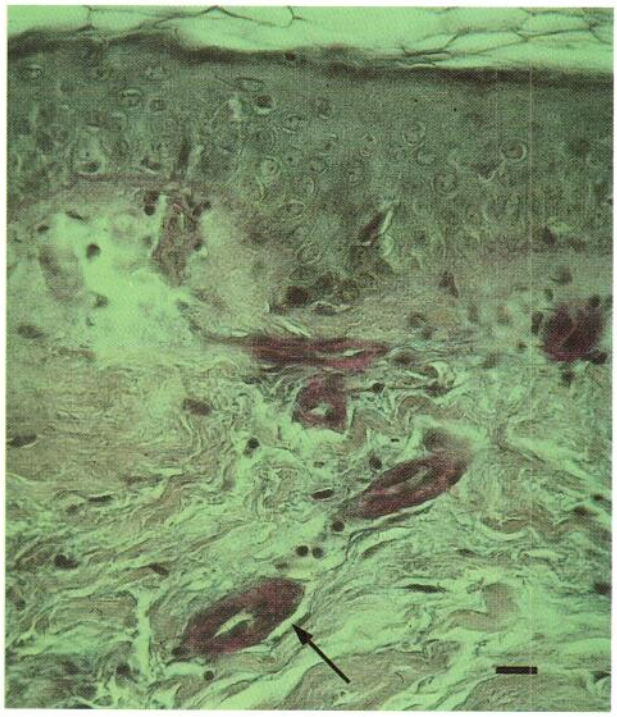

Fig. 2. Thick multilayered pericapillary basal membrane (→) in the region of glomerulum-like distortion of capillaries in severe CVI (PAS-stain; *bar* = 16 μm).

Correlation of transcutaneous oxygen tension and histological changes. Comparison between $TcPO_2$ and histological findings in the measured areas showed oxygen pressure below 29 mmHg in all areas with a thick capillary collagen IV layer, with an average $TcPO_2$ of 13 mmHg (SEM 3.39 mmHg) (Fig. 5).

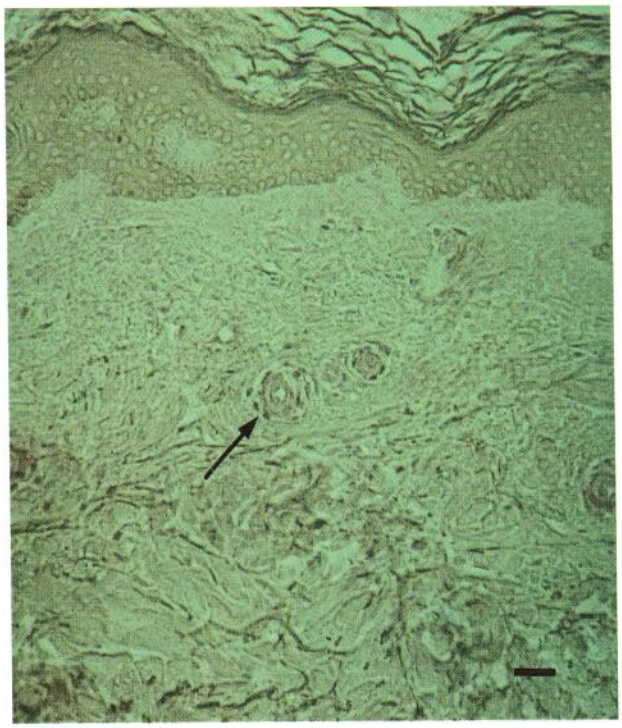

Fig. 3. Thin collagen IV layer (→) in the pericapillary basal membrane in normal skin (mAB Dako Anti-collagen IV; *bar* = 33 μm).

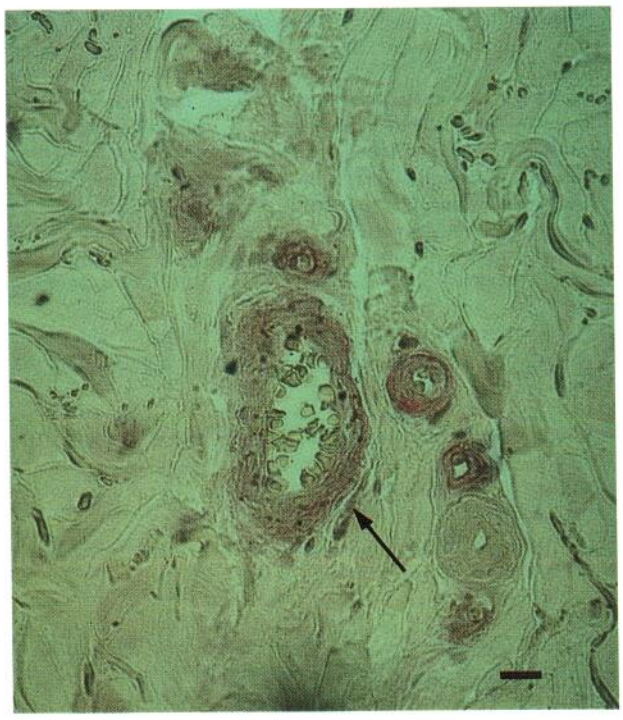

Fig. 4. Thick collagen IV layer (→) in the pericapillary basal membrane in a patient with CVI stage III (mAB Dako Anti-collagen IV; *bar* = 16 μm).

When $TcPO_2$ results from patients with medium thick collagen IV depositions were evaluated, pressures showed a wide range varying from 3 to 72 mmHg (average: 38 mmHg, SEM 6.54 mmHg) (Fig. 5).

Thin capillary collagen IV layers were correlated with high $TcPO_2$ pressures (average: 62 mmHg, SEM 4.94 mmHg) (Fig. 5).

Lowest $TcPO_2$ pressure was measured in 12 patients of CVI III and 2 patients of CVI I with intravascular microthrombi in the upper part of the corium. When microthrombi occurred, $TcPO_2$ was always below 17 mmHg (average: 6 mmHg, SEM 4.6 mmHg).

DISCUSSION

There is still discussion concerning the significance and composition of the pericapillary halo in CVI. For over a decade the pericapillary halo has been considered to be a fibrin cuff (5–7). However, other reports showed a significant increase of the collagen IV layer around capillaries (9). In this study, these results were confirmed, by demonstrating that the pericapillary halo in severe CVI actually represents a collagen IV deposition. Further, we have shown that during prolonged disease progression, especially in CVI stage III patients who had a disease history of more than 12 months, collagen IV depositions were highly increased, correlating with low $TcPO_2$.

Endothelial cells as well as fibroblasts are involved in abluminal synthesis of perivascular matrix (13). Both cell types are responsible for type IV collagen synthesis, possibly overproducing this extracellular matrix protein in an effort to limit hypoxia-induced tissue damage, thereby, however, further impeding O_2 diffusion (14). Several theories try to explain why $TcPO_2$ changes with severity of CVI. The thesis that pericapillary fibrin cuffs are responsible for the O_2 diffusion block could not be confirmed by high $TcPO_2$ measurements in areas with fibrin cuffs, detected in other studies (8). Furthermore, skin half-clearance time of Xenon, which has similar diffusion characteristics as oxygen, shows no significant difference between patients with lipodermatosclerosis and controls (15).

In this study, $TcPO_2$ was measured prior to taking biopsies. Our results show that thick collagen IV layers are associated with decreased $TcPO_2$. However, the fact that medium thick collagen IV layers were associated with pressures ranging between 3 and 72 mmHg makes the association less likely.

The lowest $TcPO_2$ tension was found in patients with microvascular thrombi, confirming the thesis that disturbed capillary perfusion might be a more important factor for the induction of tissue hypoxia (10). All patients who presented with microthrombosis also showed epidermal acanthosis. Acanthosis, however, was diagnosed in 4 cases in connection with a high $TcPO_2$ of 49 mmHg.

In view of this data it has to be discussed whether $TcPO_2$ is actually representative for the changes of the corium. $TcPO_2$ consists of the difference of two parameters: intracapillary O_2 pressure (PO_2) and PO_2 decrease over the stratum papillare and the epidermis (16). Therefore, the following additional O_2 diffusion barriers have to be taken into account: in addition to an increased collagen IV layer or a thickened basal membrane an acanthotic epidermal layer could be a possible cause for a diffusion block.

In conclusion, reduced $TcPO_2$ in CVI is certainly due to multiple factors. Besides microvascular thrombosis, a thickened basal membrane, an increased collagen IV layer and an acanthotic epidermis influence $TcPO_2$.

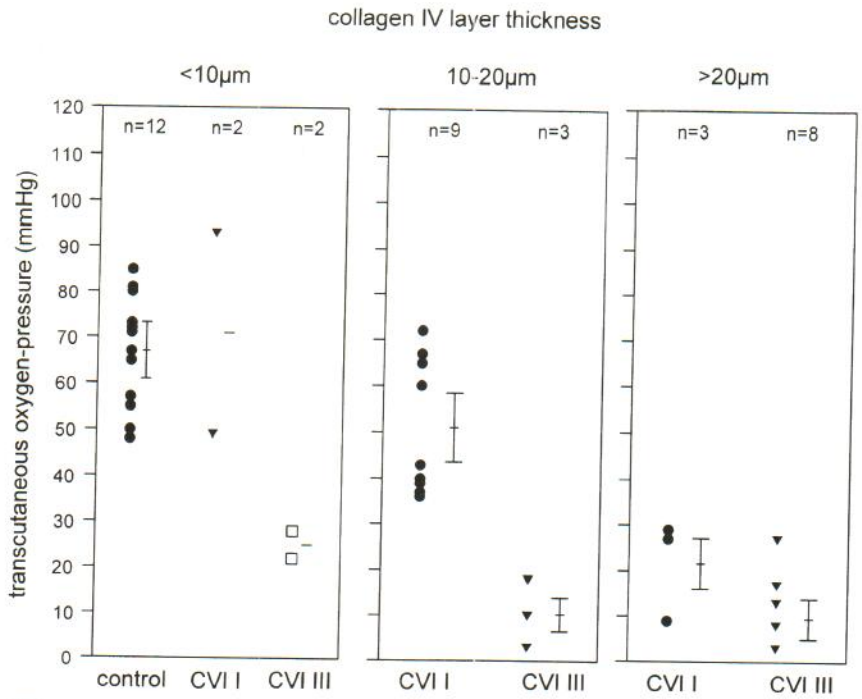

Fig. 5. Increased collagen IV layer thickness results in decreased transcutaneous oxygen pressure. In healthy volunteers and in patients with CVI stage I and III, the thickness of the collagen IV layer was determined in immunohistochemically stained biopsies and correlated to $TcPO_2$ measured prior to biopsy ($TcPO_2$ in mmHg $\pm$ SD).

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