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Transcapillary Forces and the Development of Oedema in the Lower Limb of Patients with Chronic Critical Limb Ischaemia (CLI)

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Objective: *factors regulating transcapillary fluid transport were investigated to elucidate the causes of oedema in CLI.*
Material: *sixteen patients, 6 men and 10 women (mean age of 79 ± 10.3 years) with unilateral CLI and peripheral pitting oedema.*
Methods: *measurements were performed in both limbs. Interstitial fluid was collected by applying blister suction cups on the dorsolateral part of the foot and colloid osmotic pressure of this fluid (COP_{if}) was measured in a colloid oncometer. Plasma colloid osmotic pressure (COP_{pl}) was obtained from venous blood. Interstitial fluid pressure (P_{if}) was measured by wick-in-needle technique.*
Results: *mean COP_{if} in the limbs with CLI was 2.3 S.D. 0.5 mmHg, significantly lower than in the limbs without CLI (3.1 S.D. 0.7 mmHg, $p<0.0001$). Mean COP_{pl} was 21.1 S.D. 1.8 mmHg, which was lower than in healthy controls. Mean plasma albumin concentration was 30 S.D. 6 g/l which was lower than the reference values. Mean P_{if} in the limbs with CLI was 0.7 S.D. 1.6 mmHg, significantly higher than in the limbs without CLI (−1.4 S.D. 1.4 mmHg, $p<0.0001$). The calculated mean reabsorption pressure (P_r) in the limbs with CLI was 19.6 S.D. 1.7 mmHg, significantly higher than in the contralateral limbs (16.7 S.D. 2.1 mmHg, $p<0.001$).*
Conclusion: *a low plasma albumin concentration in patients with CLI agrees with the reduction in COP_{pl} but cannot explain the oedema formation, since it is unilateral. The high P_r may cause a high transcapillary filtration pressure, resulting in a relatively great net filtration and subsequent oedema formation.*

Key Words: Chronic critical limb ischaemia; Oedema; Colloid osmotic pressure; Interstitial fluid pressure; Blister suction technique; Wick-in-needle technique.

Introduction

Chronic critical limb ischaemia (CLI) is characterised by severely diminished perfusion of nutritive capillaries and insufficient tissue oxygenation in the lower limb.[1] A substantial number of patients with CLI have distal leg and foot "pitting" oedema.[2] The effect of this oedema on the clinical course has not been clarified; it may decrease oxygenation of the tissues, thereby worsening local ischaemia.[3] A better understanding of the pathogenesis of this oedema may have therapeutic consequences.[4,5] In a previous study this oedema was shown to be significant and not caused by deep-venous thrombosis (DVT).[2] Furthermore, investigations with computed tomography (CT) demonstrated that the oedema is most pronounced distally in the limb, and mainly in subcutaneous tissue.[6] Since the predominance of the oedema was greatest where ischaemia was most severe, this oedema may indicate the severity of ischaemia.[3,6]

Oedema is generally due to an imbalance in the regulation of transcapillary fluid transport.[7–9] Fluid exchange between the intra- and extravascular space takes place across the capillary wall. This structure is regarded to be semipermeable: impermeable to plasma proteins and freely permeable to water and low molecular solutes. The interstitial fluid volume (IFV) is normally kept within narrow limits. Oedema is likely to be due to an imbalance in the hydrostatic and colloid osmotic forces across the capillary wall, resulting in net transcapillary filtration exceeding lymphatic flow (Starling, 1896). Net transcapillary filtration (F) is described by the equation:

$$F = CFC[(P_c - P_{if}) - \sigma(COP_{pl} - COP_{if})] = J_l \qquad (1)$$

where CFC is the capillary filtration coefficient. P_c and P_{if} are the hydrostatic pressures of the capillaries and interstitial fluid, respectively. Sigma (σ) is the capillary

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Table 1. Summary of patient characteristics.

Variable	Number (per cent)
Rest pain	16 (100%)
Ischaemic skin ulcer	4 (25%)
Gangrene	12 (75%)
Signs of infection	0
Heart disease	11 (69%)
Hypertension	10 (62%)
Stroke	3 (19%)
Pulmonary disease	1 (6%)
Diabetes	2 (12%)
Smoking	14 (87%)

reflection coefficient and COP_{pl} and COP_{if} are the colloid osmotic pressures of plasma and interstitial fluid, respectively.[7,9] For a capillary exchange system that is impermeable to proteins, $\sigma = 1$. If proteins are freely permeable, no osmotic pressure gradient is created and $\sigma = 0$. In subcutaneous tissue σ is probably between 0.9 and 1.0.[10–12] The interstitial fluid volume is normally kept fairly constant. This implies that lymph flow (J_l) balances net transcapillary filtration (F).

The force opposing filtration (reabsorption pressure, P_r), derived from eq. 1, is defined as:

$$P_r = \sigma(COP_{pl} - COP_{if}) + P_{if} \quad (2)$$

At no net filtration, i.e. when filtration equals reabsorption lymph flow is negligible, and therefore P_r is equal to capillary pressure (P_c).[9,10,13]

The purpose of the present study was to investigate factors regulating transcapillary fluid transport, to elucidate the pathogenesis of oedema formation in critically ischaemic limbs.

Patients

CLI was defined according to the Second European Consensus Document on CLI (1991)[14] by either of the following two criteria: persistently recurring ischaemic rest pain requiring opiate analgesia for more than two weeks, or ulceration or gangrene of the foot or toes; either of these clinical entities combined with an ankle systolic pressure ≤ 50 mmHg or toe systolic pressure ≤ 30 mmHg. Sixteen patients with unilateral CLI and peripheral pitting oedema were included. There were 6 men and 10 women with a mean age of 79 s.d. 10 years. All patients had been hospitalised for several days to receive optimal analgesic treatment. Thus, they did not need to lower their limbs for pain relief, which possibly could have played a role in oedema formation. They were either ambulant or lying in bed with both lower limbs in the horizontal position. The summary of patient characteristics is given in Table 1.

Twelve (75%) patients were on antiplatelet therapy, whereas 4 (25%) used warfarin. None of the patients had a significant DVT as assessed by colour duplex ultrasound (CDU) and venous occlusion plethysmography (VOP), described earlier.[2] Patients with an amputated contralateral limb, previous vascular surgery, bilateral CLI and oedema, clinical signs of congestive heart failure, venous insufficiency or lymphatic oedema were excluded from the study. We intended to have a control group with the same mean age and with unilateral chronic critical limb ischaemia (CLI) but *without* oedema. The "blister suction technique" was used to collect interstitial fluid for measurement of colloid osmotic pressure, COP_{if}. This technique, in our experience, is difficult to perform in limbs with CLI *without* oedema. The skin in the distal part of the lower limb in patients with CLI is fragile, and to our knowledge no other comparable *non-invasive* technique is available to collect interstitial fluid. Therefore, the contralateral limbs in patients with unilateral chronic critical limb ischaemia (CLI) with oedema were used as the control group.

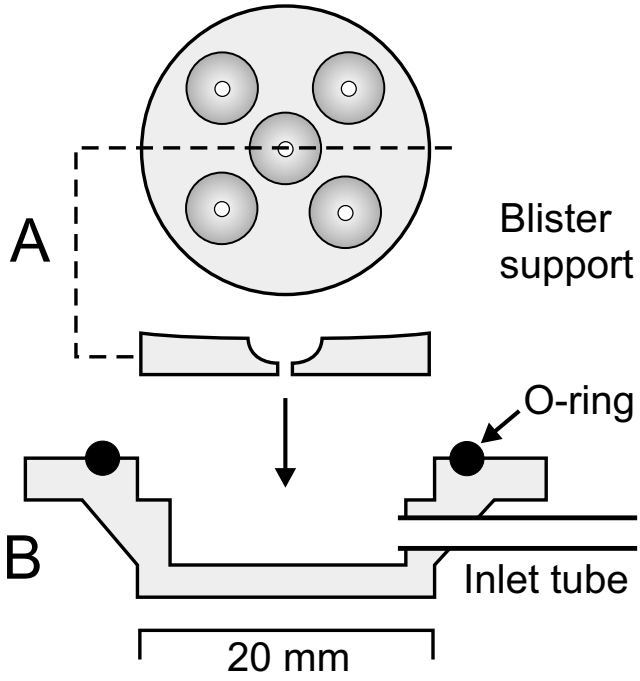
Colloid osmotic pressure of plasma in patients was compared with that of a control group, 7 women and 5 men with a mean age of 43 s.d. 10 years. They had no clinical signs of peripheral atherosclerosis, and ankle–brachial blood pressure index (ABPI) greater than 0.9. The study was accepted by the Regional Ethical Committee of Southern Norway. All patients gave their written consent.

Methods

To classify the patients as having CLI, ankle and brachial pressures were measured with ultrasound Doppler technique. In the limbs with CLI where Doppler signals were not detectable, ankle pressure was defined as 15 mmHg.

Interstitial fluid colloid osmotic pressure (COP_{if}) measurements

Interstitial fluid was collected by the blister suction technique as described by Kiistala and Mustakallio.[15] The blister suction devices were applied on the dorsolateral part of the foot (Fig. 1). Subatmospheric pressure was obtained by a manually working pump ("blister suction device", E. Stranden) with two suction cups of PVC bordered by a rubber O-ring to ensure an airtight connection between the cups and the skin (one suction cup on each foot). The 20-mm-wide suction cups each had five concave holes of 5 mm each into



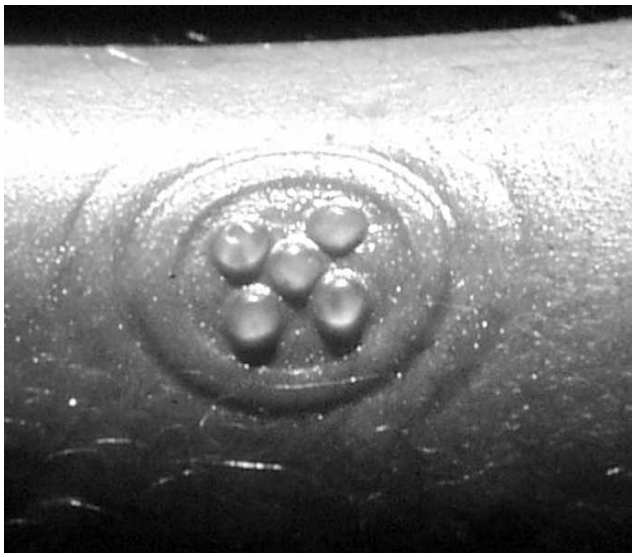


Fig. 1. Schematic illustration of a suction cup (B) with the tubing connection to the "blister suction device". (A) is the PVC diaphragm supporting the blisters. On the bottom, suction blisters developed on skin surface.

which the blisters were formed. The blisters appeared after 60–90 minutes of suction with a subatmospheric pressure of 200 mmHg.

After puncture with a thin needle, the blister fluid was collected in ordinary, unheparinized glass capillaries. The fluid samples were usually approximately 20 µl. The blister fluid colloid osmotic pressure was then measured by the colloid oncometer modified from Aukland and Johnsen[16] ("5 µl oncometer", E. Stranden). The oncometer included a dialysing membrane with a protein cut-off at molecular weight of 30 000 Dalton (Diaflo, ultrafilters, PM 30, Amicon, Lexington, Mass., U.S.A.) whereby proteins of greater size became osmotically active in the oncometer. Colloid osmotic pressure was determined with an accuracy of 0.2 mmHg. The blisters consisted of a clear, uncoloured fluid, and no samples had to be discharged because of blood contamination. Two patients had to be excluded because blisters did not develop after 90 minutes.

Blood sampling and plasma colloid osmotic pressures (COP_{pl})

Blood from antecubital veins was collected for analysis of albumin and total protein. Colloid osmotic pressure of plasma was measured by the oncometer technique described above.

Interstitial fluid hydrostatic pressure (P_{if})

Interstitial pressure was measured by the "wick-in-needle" technique on the dorsal part of the foot.[17–19] The method is based on fluid equilibrium between a pressure transducer and the interstitium. Hypodermic needles (0.8 mm OD, 40 mm length) were provided with a 4-mm side-hole approximately 7 mm from the tip (Fig. 2). The needles were filled with cotton thread and sterilised by autoclave. The thread provided a continuous water connection between tissue and needle lumen. The needle was connected to a pressure transducer (Statham P 23 Db) via a polyethylene tube (Portex manometer line, 60 cm, 200/490/060). The catheter/transducer system was filled with sodium chloride solution (9 mg/ml). To prevent leakage, the original dome was replaced by a perspex dome with a single outlet and a rubber O-ring as a seal against the transducer. The pressure transducer had a volume displacement of 0.04 µl/100 mmHg, and compliance of the total measuring system was 0.7 µl/100 mmHg. The pressure measurements were recorded with a Watanabe Mark VII linear recorder.

The connection between the tissue and transducer was checked by tightening a screw clamp on the polyethylene tube, thereby increasing the pressure. If the pressure increase was transient and returned to pre-clamp level within 2–3 minutes, the measurement was accepted. A pressure elevation that prevailed for

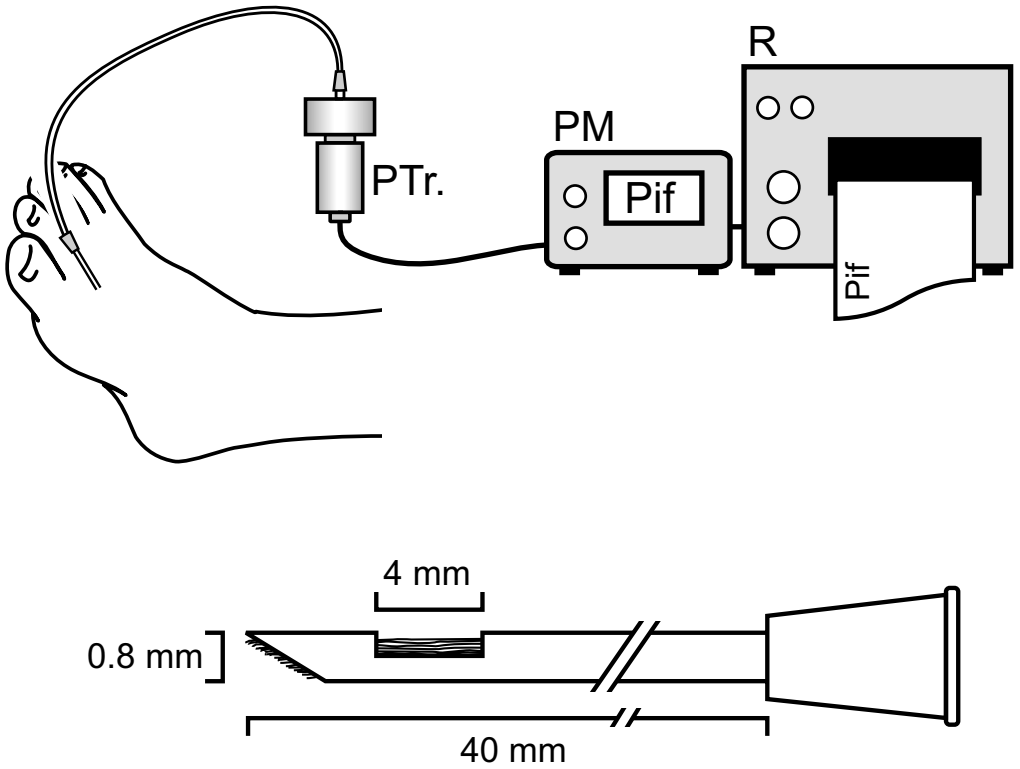


Fig. 2. Schematic illustration of the interstitial fluid pressure (P_{if}) recording system ("wick-in-needle" technique) on the dorsal part of the foot. The needle is shown in high magnification below. PTr: pressure transducer, PM: pressure monitor, R: pen recorder.

a longer period of time indicated obstruction of the needle. The system was adjusted to zero pressure by levelling the needle at the site of measurement and adjusting the pressure amplifier until zero pressure was obtained. After each recording zero pressure was checked by placing the needle at the same level. To prevent dehydration of the wick during the zero-level procedure, it was moistened with drops of sodium chloride solution (9 mg/ml).

Statistics

Wilcoxon matched-pairs signed-ranks test for independent samples was used for comparing changes in ABPI, ASP, COP_{if}, P_{if} and P_r. The results were presented as mean and standard deviation (S.D.) with $p<0.05$ as the level of significance. GraphPad Prism 3.0 was used for analysis and presentation of data (GraphPad Software, Inc., CA, U.S.A., www.graphpad, com).

Results

The mean ABPI in the limbs with CLI was 0.29 S.D. 0.15 with a mean ASP of 46 S.D. 27 mmHg, compared to 0.72 S.D. 0.31 and 114 S.D. 58 mmHg respectively for the limbs without CLI (both $p<0.001$). In 6 limbs with CLI, ultrasound Doppler signals could not be detected, and distal ankle pressure defined as 15 mmHg. The mean COP_{if} in the limbs with CLI was 2.3 S.D. 0.5 mmHg, significantly lower than in the limbs without CLI (3.1 S.D. 0.7 mmHg, $p<0.0001$, Fig. 3). The mean COP_{pl} was 21.1 S.D. 1.8 mmHg, significantly lower than in healthy controls (25.8 S.D. 1.6, $p<0.02$).

The mean plasma albumin concentration was 30 S.D. 6 g/l which was lower than the reference values at our hospital (37–48), while the mean of total protein concentration was normal. The mean P_{if} in the limbs with CLI was 0.7 S.D. 1.6 mmHg, significantly higher than in the limbs without CLI (-1.4 S.D. 1.4 mmHg, $p<0.0001$, Fig. 4). The calculated mean reabsorption pressure (P_r) in the limbs with CLI was 19.6 S.D. 1.7 mmHg, significantly higher than contralateral limbs without CLI (16.7 S.D. 2.1 mmHg, $p<0.001$, Fig. 5). For simplicity, σ is assumed to be 1.0. There were no correlation between ankle systolic pressure and P_r for either legs ($p>0.05$).

Discussion

Oedema formation is a frequent finding in limbs with critical ischaemia. Since this oedema may aggravate

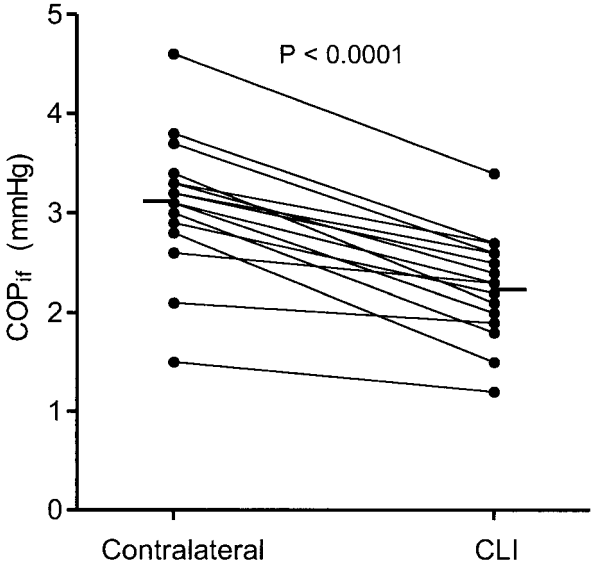


Fig. 3. The colloid osmotic pressure (COP_{if}) in the subcutaneous tissue in the limbs with CLI and contralateral limb. Horizontal bars indicate mean values.

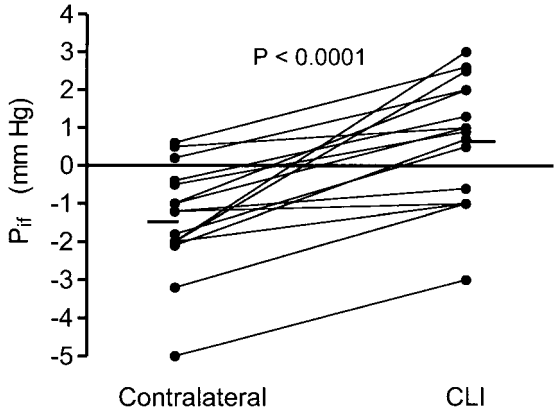


Fig. 4. The hydrostatic pressure (P_{if}) in the subcutaneous tissue measured in the limbs with CLI and contralateral limb. Horizontal bars indicate mean values.

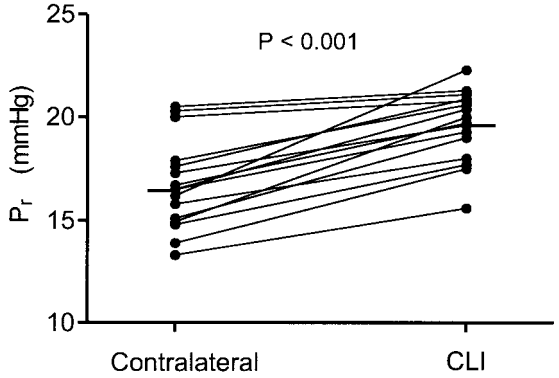


Fig. 5. The calculated reabsorption pressure (P_r) in the subcutaneous tissue in the limbs with CLI and contralateral limb. Horizontal bars indicate mean values.

ischaemia, it is relevant to gain information on its pathophysiology. The present study showed that the colloid osmotic pressure in subcutaneous tissue (COP_{if}) in the limbs with CLI and oedema was significantly lower than in the contralateral side. COP_{if} is mainly a functional expression of proteins in the interstitial fluid.[9] The capillary wall is semipermeable to proteins, depending on their molecular weight and the ultrastructural characteristics of the capillary wall itself. Although there is a unidirectional transport of plasma proteins from blood to interstitium,[20] the concentration of proteins in interstitial fluid is always less than in plasma.[21] This is primarily due to a continuous transport of protein by the lymph vessels. There is an inverse relationship between capillary filtration rate and interstitial protein concentration.[9] The finding of a reduced COP_{if} in these patients may be explained by an increased net transcapillary filtration which dilutes the interstitial fluid and promotes "wash-out" of proteins through the lymphatics.

The colloid osmotic pressure in plasma (COP_{pl}), as well as plasma albumin concentration, was low in the patients of this study. It is a general physiological phenomenon that colloid osmotic pressure is related to plasma albumin concentration. Since albumin comprises approximately half of the plasma protein mass and has a relatively low molecular weight, it is responsible for more than two-thirds of COP_{pl}.[21,22] A low plasma albumin concentration in these patients reduces the transcapillary colloid osmotic gradient and could increase the net fluid filtration across the capillary wall towards the interstitial space leading to oedema formation.[23] However, low plasma albumin concentrations in patients with CLI alone cannot explain the *unilateral* oedema formation, although it may promote oedema formation in general. The majority of patients in this study were elderly patients, many of whom are not able to provide adequate nutrition for themselves at home.[24] Therefore, malnutrition is probably the major cause of low plasma albumin concentration in patients with CLI.[25]

The interstitial fluid pressure in the subcutaneous tissue (P_{if}) at the dorsum of the foot was significantly increased (mean 2 mmHg) in the limbs with CLI. This reduces net hydrostatic filtration pressure, which in turn counteracts additional oedema formation. However, P_{if} never exceeded 4.5 mmHg, even in patients with pronounced oedema. This confirms earlier findings of a high compliance in subcutaneous tissue.[6,19] The calculated reabsorption pressure (P_r) in the severely ischaemic limbs with oedema was greater than in the contralateral sides. This is in accordance with previous findings in patients with lower-limb atherosclerosis at our laboratory.[26]

Assuming no net capillary filtration occurs, i.e. that filtration equals reabsorption, P_r is equal to capillary pressure (P_c).[9] If so, the finding of an enhanced P_r indicates an increased P_c in the ischaemic limbs with oedema. This assumption presupposes no lymph flow ($J_l = 0$), which may, however, be an underestimation. The patients may be in a state where oedema is developing, with net transcapillary filtration. In that case, P_c is likely to be higher than P_r. The difference between the two parameters is normally small, probably in the order of 0.5 mmHg under normal physiological conditions.[9] When net filtration increases, the underestimation of P_c is larger.

It is not unreasonable to assume that the reduced arterial pressure in limbs with CLI leads to a low capillary pressure. There was, however, no correlation between ankle systolic pressure and P_r. In fact, P_r in CLI was higher than in the contralateral leg. This probably reflects a reduced precapillary resistance,[27] with the result that a larger proportion of the arterial pressure is transmitted to the capillaries. Patients with CLI normally relieve their pain by lowering the ischaemic limb. Several studies have shown that the venoarteriolar response (VAR) is abolished in patients with CL1,[28–30] and therefore normal arteriolar vasoconstriction during dependency is not present.[31] This may increase foot perfusion considerably.[32] The combination of reduced COP_{if} and increased P_{if} in the ischaemic limbs reduces net transcapillary filtration pressure, thus counteracting further oedema formation.[33] On the other hand, the low COP_{if} and increased P_{if} means that the potential for *further* decrease in filtration pressure is limited.

Impaired lymph transport may contribute to unilateral lower-limb oedema. However, none of the patients had undergone previous operative procedures that could have damaged lymph nodes or vessels. Furthermore, a previous study demonstrated a normal pumping mechanism of the lymphatics ("lymph hearts") in patients with severe peripheral atherosclerosis.[34] A "chronic inflammatory process" should also be regarded as a possible explanation for oedema formation. Interactions between blood constituents and microvascular endothelial cells regulate the permeability through through the capillary wall.[35] Ischaemia might activate endothelial cells, platelets and leukocytes to secrete potent metabolites, leading to increase microvessel permeability. In addition, previous research has shown the significance of intracapillary leukocyte "sludging" during low-flow ischaemic states.[36,37] This may initiate a cascade of reactions where the formation of oxygen-free radicals plays a role.[38,39] The resulting local inflammatory process may cause vasodilatation, increased capillary permeability and oedema formation.[40]

Several methods have been introduced to collect interstitial fluid for measurement of COP_{if}. At our laboratory we have used both the "wick technique" (WT) and the "blister suction technique" (BT). Several reports have concluded that the blister suction fluid from normal skin is similar to subcutaneous interstitial fluid.[41] An underestimation by BT is to be expected, however, because of the protein-poor ultrafiltrate created by the subatmospheric pressure. COP_{if} measured by BT was found to be approximately 2 mmHg lower than obtained by the WT.[42] The blister suction technique is simple and has the advantage of being non-invasive. The blisters occur at the dermoepidermal junction between the epidermis and the basal cell membrane, without causing major alterations in the viability of the epidermis or capillary wall permeability, as long as the suction pressure is kept below 200 mmHg.[43] In our study two patients had to be excluded because blisters did not develop after 90 minutes. This was probably due to the thickness of foot skin in these particular patients. Perhaps a longer suction time and/or higher suction pressure could solve the problem, but our experience is that increased pressure can lead to blood contamination of samples. The skin in the distal part of the lower limb in patients with CLI is fragile. However, none of the patients participating in this study complained of discomfort when we applied the blister suction technique. Therefore we conclude that the method is harmless, reliable and simple.

In conclusion, a reduced plasma albumin concentration in the patients of the present study can explain the reduction in COP_{pl} but not the *unilateral* oedema formation. The reduced COP_{pl} and increased P_r indicate an increased transcapillary filtration pressure, promoting oedema formation. The resulting enhanced transcapillary transduction of fluid will affect the interstitium by dilution and lymphatic "wash-out" of interstitial proteins (lowering COP_{if} as well as elevating tissue pressure). On the other hand, the low COP_{if} and increased P_{if} means that the potential for preventing *further* oedema formation is limited.

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