The use of microcirculatory techniques in the assessment of pathophysiology, diagnosis and management of critical limb ischemia

de Graaff, J.C.

Citation for published version (APA):
de Graaff, J. C. (2003). The use of microcirculatory techniques in the assessment of pathophysiology, diagnosis and management of critical limb ischemia

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 1
General introduction

Peripheral arterial disease and critical limb ischemia

Arteriosclerosis is a generalized cardiovascular disease. It is a process of thickening of intimal and medial layers of arteries and arterioles causing stenosis and obstruction, which may result in widespread symptoms, such as myocardial, cerebral or peripheral ischemia. Peripheral arterial disease (PAD) is due to arteriosclerosis of the arteries supplying the lower leg. The symptoms of PAD are caused by a shortage of local microcirculatory perfusion of muscles and skin as a result of insufficient arterial blood supply.

In the European realm of vascular medicine, the clinical symptoms of PAD are usually classified according to Fontaine. Intermittent claudication (IC; Fontaine stage II) is the mildest form of PAD, and is characterized by exercise-induced leg pain (usually located in calf, thigh, or hip), which disappears after the patient stops exercise. Intermittent claudication can be treated conservatively (walking exercise and secondary prevention) or by vascular intervention (percutaneous transluminal angioplasty [PTA] and/or vascular surgery). Critical limb ischemia (CLI) is the end stage of PAD. In CLI, PAD has progressed to such an extent resulting in ischemic pain in the foot at rest (Fontaine stage III) or breakdown of skin (presenting as an ulcer or gangrene; Fontaine stage IV). In these patients a revascularization procedure is the therapy of choice to treat the serious symptoms and to avoid amputation.

Epidemiology

CLI is a common manifestation of cardiovascular disease, because atherosclerosis is a generalized disorder with a high incidence in Western society. Cardiovascular diseases are the main cause of death (35.5%) in the Netherlands. The prevalence of PAD in subjects aged 55 years and over was found to be 19.1% in the Rotterdam (ERGO) study. Symptoms of IC were reported by only 1.6% in the same population. The incidence of CLI has never been investigated directly, but has been estimated to be 300 per million per year (5% of the patients with IC develop CLI within 5 years).

Common risk factors for the development and progression of PAD are smoking (relative risk, RR = 1.7), diabetes mellitus (DM; RR = 3.3), hypercholesterolemia (RR = 2.0), and hypertension (RR = 2.8). Because of the generalized character of arteriosclerosis, PAD is frequently associated with other cardiovascular diseases, such as coronary artery disease (CAD: 40-90%) and cerebrovascular disease (CVD: 26-50%).

In 2000, arterial disease (non-cardiac) accounted for only 2105 primary cause of deaths in the Netherlands (1% of all death). The contribution of CLI per se is not known, but is considerably lower since these figures also include mortality caused by aortic aneurysms and peripheral arterial thrombosis. However, patients with CLI have a severely reduced life expectancy, with a 26% one-year mortality rate increasing to 56% after five years. The majority of these deaths is caused by cardiac diseases (52%).

Chapter 1 - General introduction

Standard techniques to investigate PAD

Chronic ischemic rest pain, ulcers or gangrene are classic symptoms of critical limb ischemia, but are not specific. Rest pain could also be caused by diabetic neuropathy or compression of the spinal roots, while persisting ulcers could be caused by venous insufficiency and diabetes mellitus. Therefore, additional objective diagnostic procedures are required to show and quantify vascular insufficiency. The techniques presently used for routine investigation can be divided into two categories: techniques that provide functional information about the presence and severity of the diseased peripheral tissue (mainly ankle blood pressure measurements) and techniques that provide more or less anatomical information about the location of the obstruction (e.g. duplex scanning, arteriography, magnetic resonance angiography), necessary to define type and location of vascular intervention.

Functional information

Ankle blood pressure

The most simple and frequently used functional test is the Doppler-derived systolic ankle blood pressure. The AP reflects the overall occlusive process in the arteries supplying the legs. The AP is a simple, non-invasive investigation and is used as a screening test for the presence of PAD. However, the AP is not related to the need for and outcome after vascular intervention, risk of amputation, and wound healing in patients with severe PAD. Furthermore, the AP is unreliable in a considerable number of patients (about 25%) because of presence of incompressible arteries by medial calcification (Monckeberg’s sclerosis), as occurs in patients with diabetes mellitus (80% of patients with PAD and DM have incompressible arteries), use of corticosteroid therapy, longstanding renal disease, and after kidney transplantation.

Toe pressure

The toe pressure (TP) is also a simple non-invasive test and provides functional information of the arteries of the leg down to pedal and digital arteries (figure 1). In patients with severe PAD, the TP is correlated with the severity of the overall occlusive process, need for vascular intervention, healing potential of ulcers, and prediction of the need for amputation. Furthermore, the TP is much less influenced by medial calcifications than the AP. Despite the apparent advantages of TP as compared to AP, the TP is not commonly used in clinical practice.

Figure 1.
Example of toe blood pressure measurement (left panel) with registration of the reappearance of the blood flow during deflation of the manchet (right panel).
Figure 2. Anatomical chart constructed after duplex scanning (left panel) and corresponding angiography (right panel) of the arteries of the lower leg with CLI.

**Anatomical information**

Arteriography, duplex scanning, and magnetic resonance angiography (MRA) are techniques to localize and grade hemodynamically significant obstructions (figure 2). However, the site of the obstruction per se is not related to the severity of symptoms and should therefore not be used as a single tool in the diagnosis of CLI. Furthermore, these techniques are time consuming and require specialized physicians. They have a limited interobserver agreement (arteriography: kappa = 0.3-0.7, duplex: kappa = 0.4 - 0.8) especially in more distal segments, which are mostly affected in patients with CLI. Additionally, arteriography is an invasive technique and may have side effects like bleeding complications and anaphylactic reactions to contrast medium (2% of the procedures).

**Classification of PAD**

International classification of PAD is necessary to specify patients requiring vascular intervention, and compare and interpret adequately the results of trials. However, the definition of CLI is controversial. CLI was first defined by Jamieson in 1981 as a state of severe peripheral arterial insufficiency requiring vascular intervention to prevent from ultimate amputation. In the latest consensus (the Transatlantic Inter-Society Consensus (TASC) on the Management of Peripheral Arterial Occlusive Disease, 2000) it is stated that “the term CLI should be used for all patients with chronic ischemic rest pain, ulcers, or gangrene attributable to objectively proven arterial disease.” Therefore objective criteria to quantify PAD appear to be necessary for the definition of CLI. In the last decades several objective criteria, mainly based on ankle pressure, have been proposed (mainly European and North American standards, see table 1). The criteria have been criticized by many, mostly proclaiming that the criteria were too strict. The presented cut-off levels for the AP are admittedly arbitrary, and it is recognized that no single level can clearly separate categories.
<table>
<thead>
<tr>
<th>Fontaine SVS/ISCVS grade</th>
<th>SVS/ISCVS Clinical symptoms</th>
<th>SVS/ISCVS criteria</th>
<th>ECD criteria</th>
<th>TASC</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>asymptomatic - no hemodynamically significant occlusive disease</td>
<td>Normal treadmill or reactive hyperemia test</td>
<td>NS</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>mild claudication</td>
<td>Completes treadmill† exercise; AP after exercise &gt; 50 mm Hg, but &lt; 20 mm Hg lower than resting value</td>
<td>NS</td>
</tr>
<tr>
<td>I</td>
<td>2</td>
<td>moderate claudication</td>
<td>Between categories 1 and 3</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>3</td>
<td>severe claudication</td>
<td>Cannot complete treadmill† exercise and AP after exercise &lt; 50 mm Hg</td>
<td></td>
</tr>
<tr>
<td>III* II*</td>
<td>4*</td>
<td>ischemic rest pain</td>
<td>Resting AP &lt; 40 mm Hg, flat or barely flat pulsatile ankle or metatarsal PVR; TP &lt; 30 mm Hg</td>
<td>AP &lt; 50 mm Hg and/or TP &lt; 30 mm Hg</td>
</tr>
<tr>
<td>IV* III*</td>
<td>5*</td>
<td>minor tissue lost (ulcers or gangrene)</td>
<td>Resting AP &lt; 60 mm Hg, ankle or metatarsal PVR flat or barely flat pulsatile; TP &lt; 40 mm Hg</td>
<td>AP &lt; 50-70 mm Hg or TP &lt; 30-50 mm Hg or TcpO₂ &lt;30-50 mm Hg</td>
</tr>
<tr>
<td></td>
<td>6*</td>
<td>major tissue loss-extending above TM level, functional foot no longer available</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AP: ankle pressure;  
PVR: pulse volume recording;  
TP: toe pressure;  
TM: transmetatarsal;  
NS: not specified;  
SVS: Society for Vascular Surgery;  
ISCVS: International Society for CardioVascular Surgery;  
ECD: European Consensus Document;  
TASC: TransAtlantic Inter-Society Consensus, management of peripheral arterial disease  
*: Fontaine stages III and IV, and SVS/ISCVS Grades II and III (categories 4, 5 and 6) are embraced by the term chronic critical limb ischemia;  
†: five minutes at 2 mph (3.2 km/h) on a 12% incline.
Two independent definitions are proposed in the latest TASC consensus.\textsuperscript{2} The clinical purposes of the definition of CLI incorporates the classification and identification of patients needing a vascular intervention. The definition of CLI is strictly based on clinical symptoms attributable to objectively proven arterial disease, whereas for research purposes the TASC advises “the aim being to be ensure that the ulceration, gangrene or rest pain is indeed caused by peripheral arterial disease and that most would be expected to require major amputation within the next 6 months to a year in the absence of a significant hemodynamic improvement.” Furthermore, the consensus suggested for research purposes to use absolute pressures of either AP (< 50-70 mm Hg), TP (< 30-50 mm Hg) or $\text{TcPO}_2$ (< 30-50 mm Hg).\textsuperscript{2}

**Indication for vascular intervention**

As accurate criteria to quantify PAD are lacking, there is a wide variation as to the amount of diagnostic procedures and indication for vascular intervention among hospitals and countries.\textsuperscript{21,31} Previous reports showed that CLI may well exist despite an ankle pressure >50 mm Hg or a toe pressure of >30 mm Hg.\textsuperscript{29,32} In a recent study the ankle blood pressure was found to have a very low predictive value (17%) as to the presence of CLI that needs invasive therapy.\textsuperscript{8} Therefore, at present, the identification of patients suspected of CLI requiring vascular intervention is mainly based on subjective criteria. The diagnosis of CLI is mostly based on a combination of AP, ankle brachial pressure index (ABPI), and the clinical view of a specialist with vascular expertise. In addition, duplex scanning and/or arteriography is frequently performed to assess the extent of the disease and to plan an intervention even before a clear indication for vascular intervention has been established.\textsuperscript{21,31}

Microcirculatory techniques provide objective functional information about the endangered tissue in patients with CLI.\textsuperscript{32,33} Previous studies have suggested that the combination of TP and $\text{TcPO}_2$ might be beneficial in the identification of patients requiring a vascular intervention. The combination of TP and $\text{TcPO}_2$ yields the best accuracy as to the need for vascular intervention.\textsuperscript{8} However, TP and $\text{TcPO}_2$ are only used rarely in clinical practice despite the potential diagnostic value of these parameters.\textsuperscript{21}

**Treatment and outcome of CLI**

Vascular intervention is the only conclusive treatment for patients with CLI to provide sufficient blood flow to relieve rest pain and heal skin lesions. This can be performed by endovascular techniques (PTA) and/or arterial surgery with vein and/or prosthetic grafts.\textsuperscript{2} The choice of therapy is a balanced one and depends on the level and severity of arterial disease, risk of anesthesia, risk of complications and potential patency of intervention. Vascular interventions have side effects like graft infection (10-30 \%) and occlusion. The primary patency at 3 years varies between 90\% (for aortobifemoral grafts) to less than 40\% (for femorodistal prosthetic grafts), with risk of amputation.\textsuperscript{2} Therefore, timing of and careful selection of patients for an intervention is extremely important.

In addition to vascular intervention, primary amputation (mostly minor amputations) is frequently (5-25\%) necessary to control infection and pain.\textsuperscript{34,35} Secondary amputation is still necessary in a group of patients with continued progression of atherosclerosis and no option for vascular intervention.\textsuperscript{37} Despite
an aggressive intervention policy, the major amputation (defined as being unable to walk without prosthesis) rate is high, and varies between 12% after 3 months\textsuperscript{35} to about 40% of the patients still alive after 6 months.\textsuperscript{2}

Other old and new (conservative) treatment modalities are in the position of last resort if reconstruction is not possible, although objectively the effect of these treatments have never been proven.\textsuperscript{2} These are, among others, hyperbaric oxygen therapy\textsuperscript{36-40} ambulant intermittent venous compression\textsuperscript{41}, epidural spinal cord stimulation\textsuperscript{2,43} (also effective in pain control), sympathectomy, and pharmacotherapy, like prostanoids, antiplatelet drugs and anticoagulants.\textsuperscript{2} More recently new promising therapeutic modalities have become available which have not been evaluated sufficiently yet: gene induced therapeutic angiogenesis (VEGF)\textsuperscript{,2,44} and autologous implantation of bone marrow-mononuclear cells.\textsuperscript{45}

Another part of therapy of CLI focuses on the control of pain, foot and wound care, and on control of risk factors for secondary prevention (smoking cessation, control of hypertension, diabetes, and hyperlipidemia).\textsuperscript{46} One of the problems with wound care and non-invasive treatment of CLI is the formation of pre and post-operative oedema. The presence of postoperative oedema is reported to vary between 40 and 100% in the legs with distal bypass procedures.\textsuperscript{47-49} The oedema causes increased intracellular distance due to distensibility of the interstitial space, which aggravate hypoxia in already critically ischemic tissues.\textsuperscript{48} However pathophysiological reasons behind this edema are virtually unknown.\textsuperscript{47,48,50}

**Impact of CLI on quality of life and medical costs**

The symptoms of CLI are intense and have a large impact not only on the diseased limb but also on patient’s health-related quality of life (HRQOL). Previous studies performed in patients with PAD mainly focused on the relation between HRQOL on one hand and the outcome and success of therapy (e.g. amputation and bypass therapy) on the other.\textsuperscript{51-53} These studies show that aggressive (infra-genicular) bypass surgery is preferred over amputation, since it is associated with a superior HRQOL.\textsuperscript{54-56} However, to improve treatment of patients with CLI one should not only focus on treatment of the diseased limb, but also on improvement of the understanding of the patient’s sufferings and needs specific to improve HRQOL. Specific knowledge about the patients sufferings and needs is lacking.

The treatment of CLI is expensive and time-consuming. The total costs (diagnosis, treatment and rehabilitation) for the treatment of CLI varies between 8000 Euro for a successful primary endovascular or bypass procedure to 35.000 Euro for a primary distal bypass procedure and 45.000 Euro for a secondary amputation.\textsuperscript{2,37,57,58} The mean admission period to the hospital of patients with clinical CLI is long (12 days).\textsuperscript{3}

**The significance of microcirculation in PAD**

The final symptoms of patients with PAD are caused by a disturbed microcirculatory perfusion distally to the macrocirculatory disorder. In patients with intermittent claudication there is a relative and temporary shortage of microcirculatory perfusion during exercise. In patients with CLI, the blood flow in nutritional capillaries decreases below the minimal demand and consequently symptoms of tissue necrosis occur: pain at rest, ulcers and gangrene.
Regulation of blood flow in the healthy skin
The cutaneous microcirculation consists of an ingenious vascular network which serves two major functions: first, nutrition of the skin (about 5% of the total skin blood flow), and second, thermoregulation (about 95% of the total skin blood flow). The superficial layer consist mainly of a nutritional network of arterioles, capillaries and veins (figure 3). The deeper thermoregulatory type consists of (a) an extensive subcutaneous venous (or subpapillary) plexus, which holds large quantities of blood that can heat the surface of the skin, and (b) arteriovenous anastomoses, which are large vascular communications directly between the arteries and venous plexus. The arteriovenous anastomoses are predominantly found in the volar surfaces of hands and feet, and not in the dorsal sides. The walls of the arteriovenous anastomoses contain strong muscular cells innervated by sympathetic nerve fibers that secrete norepinephrine to induce vasoconstriction.

The blood flow through the skin is regulated locally and centrally, and is able to vary markedly (from 50 ml - 2 liter /per minute) as a result of its structure and rich innervation. The regulation of skin blood flow is complex because the flow serves many different adaptative mechanisms such as changes in local and central temperature, posture, central blood pressure, but also changes as a result of local metabolic demands. The blood flow required for nutrition of the skin is only very small (in total about 40 ml/min). Yet, at ordinary skin temperature, the amount of blood flowing through the skin is 10 times (=0.25L/m² = 400ml/min in a normal adult) more than what is needed for the nutrition of the tissues. Furthermore, the skin serves as a reservoir for pooling of blood which can be mobilized by sympathetic activation during circulatory stress (e.g. emotion, deep breath, hemorrhage and exercise).

Most of the blood flow through the skin is controlled centrally by the nervous system to control central body temperature. The temperature control center in the hypothalamus (central regulation) is capable to regulate vasoconstriction by secretion of norepinephrine at the end of sympathetic vasoconstrictor fibers and...
vasodilation by a complex of sympathetic fibers that secrete acetylcholine and activate bradykinin.\textsuperscript{62,65}

The \textit{local} control comprise of four mechanisms (figure 4):

1. A myogenic vasoconstriction response. This is a contraction of smooth muscle cells activated upon an increase in transmural pressure in arteriolar wall induced by an increase in arterial pressure and a venous damming effect.\textsuperscript{66-69}

2. A veno-arteriolar (or veno-vasomotor) reflex. This is the vasoconstriction upon an increase in venous volume, as is present in standing position. Stretch receptors in venules, directly triggered by a venous volume increase, lead to a local, retrograde axon reflex, which causes arteriolar constriction.\textsuperscript{70,71}

3. A locally regulatory mechanism by various metabolites formed in the surrounding tissue (including $K^+$, lactic acid, $PO_2$, $PCO_2$, adenosine, ATP, and increases in osmolarity) and nitric oxide (endothelium-derived relaxation factor).\textsuperscript{72} These factors probably also cause reactive hyperemia in response to periods of ischemia.

4. A local vasoconstriction on temperature down to a temperature of about 15 $^\circ$C, probably by increased sensitivity of the vessels to nerve stimulation. At temperatures below 15 $^\circ$C the vessels begin to dilate.\textsuperscript{73,74}

In addition, there is a natural rhythmical variation in skin blood flow, called vasomotion. Normal vasomotion ensures regular, periodic perfusion of nutritive capillary networks in the skin in healthy tissue. This process is caused by spontaneous changes in arteriolar diameter by the combination of various regulatory mechanisms.\textsuperscript{75,76}
The capillary network serves the interchange of nutrients, cellular excreta and other substances between tissue and blood. The interchange to interstitial fluid happens by diffusion through the capillary membrane. The capillary blood pressure is an important component in the forces that determine the fluid exchange, which are determined according to the Starling's equation. Therefore, the capillary pressure is thought to be the crucial factor in the fluid and electrolytes balance at capillary level.

**Techniques for the investigation of the microcirculation**

**Laser Doppler**

Laser Doppler fluxmetry is a non-invasive technique to assess cutaneous microcirculatory blood perfusion. Laser light with a wavelength of 780 nm is conducted through optical fibers to the skin where it penetrates the skin to a depth of 1-1.5 mm and is reflected partly (figure 5). When backscattered by moving objects (principally erythrocytes), this light undergoes a frequency shift, which is proportional to the velocity and number of moving objects (flux), and is expressed in Volts (laser Doppler flux, LDF). LDF measures flow not only in the capillaries, but also in the subpapillary venular and arteriolar plexus and arteriovenous shunts (figure 5). The LDF signal represents mainly thermoregulatory (about 95%) and hardly nutritive (about 5%) skin perfusion. Furthermore, LDF is dependent on anatomic variables (such as skin pigmentation, thickness of epidermis) which defines the depth of penetration.

LDF is not frequently used and of limited value in the management of CLI because of its large spatial and temporal variation. At present, LDF is mostly used in the field of physiology and pharmacology in combination with provocation tests (post occlusive hyperemia, influence of venous pressure and posture and iontophoresis).

**Figure 5. Schematic impression of laser Doppler.**
transcutaneous oxygen pressure measurements

Transcutaneous oxygen pressure measurement appreciates the surplus amount of oxygen at the skin surface after diffusion from superficial skin vessels (figure 6). The partial transcutaneous oxygen pressure (TcpO$_2$) provides information about the oxygen tension of the underlying tissue, so any changes in oxygen uptake, transport or release will be reflected by TcpO$_2$. The electrode used, based on the original design by Clark$^{86}$, contains a heating element, thermostat, a platinum cathode and silver chloride anode. The electrode is surrounded by electrolyte fluid covered with a membrane permeable for oxygen. The element heats the surrounding skin and local blood perfusion to a preset temperature (mostly 44 °C). This maximizes local microcirculatory perfusion by vasodilation, shifts the oxygen dissociation curve to the right, and increases the permeability of oxygen through the skin. As a result oxygen diffuses from the blood through the skin. In this transit the oxygen is partly consumed by surrounding tissue (in normal skin approximately 30 mm Hg).$^{87}$ At the electrode the oxygen diffuses through the membrane to the cathode, where a reduction of oxygen occurs. This reduction causes a current, which is dependent on the amount of oxygen.$^{88}$

TcpO$_2$ reflects nutritive microcirculatory perfusion of the underlying skin, which is severely reduced in patients with CLI.$^{83}$ The TcpO$_2$ is a simple, non-invasive measurement with a good diagnostic accuracy in relation to the clinical need for a vascular intervention.$^{8,33,89}$ However, at present the TcpO$_2$ is not commonly used in clinical practice.$^{21}$

Capillary microscopy

Capillary microscopy visualizes and can provide information about capillary morphology, capillary density, red blood cell velocity (RBCV) and diameter.$^{83}$ Capillary microscopy visualizes only the superficial nutritive perfusion. Unfortunately, this is only possible at lips and at the nailfold (eponychium) of fingers and digits of foot, since at these sites the capillaries run parallel with the skin and can be reached in vitro in humans with the capillary microscope. Recently, a new tool, orthogonal polarization spectral (OPS) imaging, has become available which allows detailed visualization of the microcirculation of among
others the nailfold, mouth, rectum and organs during surgery (e.g. brain and abdominal viscerae).\textsuperscript{89-91}

In patients with CLI there is a reduced capillary density, a reduced red blood cell velocity (RBCV) at rest, and a reduced increase in RBCV during post-occlusive reactive hyperaemia.\textsuperscript{83,92} Furthermore, capillary microscopy is a good predictor of the need for amputation in patients with non-reconstructible leg ischemia and is used for the diagnosis of Raynaud's phenomenon.\textsuperscript{43} Nevertheless, capillary microscopy is only available in some specialized centers and is rarely applied clinically in patients suspected of CLI. Like laser Doppler and TcpO\textsubscript{2}, capillary microscopy is mostly used in research settings.

**Capillary pressure measurements**

Capillary pressure can be estimated by indirect functional measurements and by direct cannulation. The indirect methods can be subdivided into two principles: the venous occlusion method on one hand and the isogravimetric and isovolumetric methods on the other. With the latter methods, capillary pressure is estimated from an equilibrium of fluid exchange in an organ monitored by the weight or volume of the tissue.\textsuperscript{93-95} With the venous occlusion method the whole-organ capillary pressure is estimated from the inflection point of a venous pressure tracing after sudden occlusion of the venous outflow.\textsuperscript{96,97} The method has also been described for use in human limbs,\textsuperscript{98} although its use remains debatable.\textsuperscript{99}

In the direct method, the pressure is measured by direct cannulation of the capillary. In 1930 Landis cannulated the capillaries with a micropipette communicating with a manometer, the height of which was adjusted to balance the blood pressure under observation.\textsuperscript{100} However, the size of the tip of the pipette is too small to allow fast intermittent flow in order for the manometer to trace heart beats.\textsuperscript{101} Therefore Wiederhielm et al.\textsuperscript{102} designed a servo-nulling micropressure system. Hereto the micropipette, filled with a 2 molair solution, is used as an ultralow compliance transducer. The servo-nulling system balances the change in impedance, caused by an influx of blood into the pipette tip, by a counteracting pressure equal to the capillary pressure. In the commercially available system by Intaglietta et al.\textsuperscript{103} this counteracting pressure is generated by a magnetic driving unit and transmitted to the pipette through a system filled with oil.\textsuperscript{101,103} This system has only been used in humans in finger nail fold by Mahler et al.,\textsuperscript{104} and Tooke and Shore et al.\textsuperscript{78,105}, and has never been used in legs.

**Microcirculatory changes in PAD**

Stenosis and obstructions in the arteries (macrocirculation) ultimately lead to a compromised microcirculation with structural adaptations and disturbance of regulatory mechanisms. In addition, inappropriate activation of hemostasis and inflammation also play a central role in the pathophysiology of CLI.\textsuperscript{106}

Using capillaroscopy morphologic changes of the nutritional skin capillaries can be observed in endangered areas of severely diseased limbs. The morphology of capillaries at the foot of patients with intermittent claudication do not differ from healthy volunteers. Only in patients with CLI marked capillary alterations can be observed. Initially this is reflected in indistinct visualization of capillaries changing to a reduction or complete absence of the number of blood filled capillaries.\textsuperscript{32,83,92,107} This may be caused by (1) a collapse of precapillary arterioles caused by low transmural pressure, arteriolar vasospasm, abnormal vasomotion,
microthrombosis, interstitial edema, and by (2) capillary occlusion caused by endothelial cell sealing, platelets aggregates, rigid adhesive leucocytes, or blood cells-platelets aggregates.\textsuperscript{75,106} This leads to an inhomogeneous distribution of skin microcirculatory flow. Besides, there is a small reduction in capillary red blood cell velocity and an increase in capillary diameter in the supine position in severely diseased limbs.\textsuperscript{83,108} Overall, there is a reduced capillary perfusion,\textsuperscript{83} which results in a decreased transcutaneous oxygen pressure.\textsuperscript{32,109}

The persistent shortage of microcirculatory perfusion in CLI leads to structural adaptations and disturbances of regulatory mechanisms, which give rise to an increased peripheral (capillary) perfusion upon dependency. This may explain the pain relief these patients experience upon leg dependency,\textsuperscript{47,84,110-112} but might also cause (postoperative) edema formation.\textsuperscript{26,84,113,114}

Capillary hypertension is thought to be the crucial factor in the formation of edema and relief of ischemic rest pain in patients with severe PAD. Capillary pressure is generally thought to be delicately regulated by the autonomic nervous system. The peripheral vasoconstriction responses, e.g. the venoarteriolar reflex, regulate capillary pressure and prevent capillary hypertension in the leg-dependent position.\textsuperscript{59,115} Previous studies showed that capillary pressure autoregulatory mechanisms are impaired in patients with PAD in such a way that upon dependency of the limb, the normal postural vasoconstriction response that limits the increment in capillary pressure will be compromised. This will enhance transcapillary transport, which results in pain reduction in the leg-dependent position, but also to edema formation as a result of capillary hypertension and excessive filtration of fluid.\textsuperscript{26,84,114,116,117}

So far, knowledge about the blood pressure in the capillaries of foot skin has been derived either from indirect estimations or few invasive, static pressure measurements performed in two healthy volunteers.\textsuperscript{98,111,118} This pressure parameter provides important information about the relation between peripheral perfusion, pressure, and resistance.\textsuperscript{111,119} in patients with peripheral vascular disease, capillary pressure is unknown. Appreciation of the regulation of microcirculatory perfusion in various disease states is particularly important in the improvement and development of conservative remedies (vaso-active drug therapy, intermittent compression therapy, spinal cord stimulation, etc.) and operative treatment strategies, or even complications (post-operative edema formation) of PAD.\textsuperscript{43,84,92,120-121}

**Aim of the thesis**

Microcirculatory techniques may provide objective functional information about the endangered tissue in patients with CLI. Therefore, this thesis focuses on the significance of microcirculatory techniques in the pathophysiology, diagnosis and management of patients with CLI and deals with some issues about assessing the severity of CLI:

1. **What is the clinical value of microcirculatory techniques in the diagnosis and management of patients suspected of CLI?**
2. **What are the microcirculatory changes, in particular changes in capillary pressure, in patients with PAD?**
3. **What is the influence of CLI on Health Related Quality of Life (HRQOL)?**
Outline of the thesis

The toe blood pressure parameter can play an important role in the diagnosis of CLI. The routinely applied technique (photoplethysmography and strain gauge plethysmography) have shortcomings in the lower pressure range. In chapter 2 the laser Doppler technique was evaluated to investigate whether the accuracy of toe blood pressure measurements could be improved.

The AP is generally known to be a reproducible parameter. However, the reproducibility of TP and TcpO₂ is less thoroughly investigated. Therefore, AP, TP and TcpO₂ are evaluated as to their inter-observer and intra-observer variability. The results of this evaluation are described in chapter 3.

In chapter 4 we describe a study to assess the optimal cut-off values of toe blood pressure (TP) and transcutaneous oxygen pressure (TcpO₂) in the supine and sitting positions, in order to accurately detect the presence of severe leg ischemia requiring invasive treatment. The diagnostic power is evaluated by means of ROC analysis, using the clinical decision for intervention or the vascular intervention itself as a reference standard.

Despite the diagnostic advantages of TP and TcpO₂, these parameters are rarely used in clinical practice. In therapeutic research, the randomized clinical trial design is generally accepted as the ultimate type of evaluation. Therefore, we intended to evaluate the clinical value TP and TcpO₂ in a randomized clinical trial. However, the experience and knowledge of a diagnostic randomized clinical trial is sparse in literature. Thus, in chapter 5 we describe the rationale, theoretical considerations, design issues, limitations, and solutions of a randomized clinical trial in the evaluation and implementation of diagnostic tests. These ideas have been generated and developed through progressive methodological insight by our group throughout the set-up and follow-up of the study. We use the clinical problem of CLI as an example.

In chapter 6 we present the actual results of the trial described in chapter 5. We compared the diagnostic value of two management strategies in a D-RCT to identify patients requiring vascular intervention. Patients clinically suspected of CLI were randomized for either management based on AP and the clinical view of a vascular specialist or management based on TP and TcpO₂. The clinical outcome was used as endpoint parameter.

Skin microcirculatory perfusion in the foot has been studied intensively in healthy and diseased subjects by techniques, such as laser Doppler fluxmetry, capillary microscopy and transcutaneous oxygen measurements. To date, capillary pressure is unknown in patients with various stages of peripheral vascular disease, and hence, the relation between peripheral perfusion, pressure, and resistance. Therefore, we developed a technique to measure capillary pressure in fingers and feet using a servo-nulling micropressure system. The development and validation of this technique is described in chapter 7.

Thus far, capillary pressure measurements in the feet have only been performed in two healthy volunteers using a static technique. In chapter 8 we describe the regulation of dynamic capillary pressure and perfusion and effect of postural changes in the hallux in healthy subjects. With the presented set-up we are able to provide simultaneous information about the macro-circulation (ankle and toe pressure) and micro-circulatory pressure (capillary pressure, capillary pulse pressure amplitude) and microcirculatory flow parameters (RBCV and LDF).
Finally, the pathophysiology of microcirculatory changes in patients with various stages of PAD using microcirculatory techniques including capillary pressure measurements is described in chapter 9.

CLI is a limb threatening disease with severe symptoms and has a large influence on patient morbidity. Less is known about the impact of this limb-threatening disease on Health Related Quality of Life (HRQoL). The evaluation of HRQoL of patients with peripheral arterial disease in comparison with coronary artery disease is described in chapter 10.

Chapter 11 summarizes this thesis, provides a general discussion and offers suggestions for further research.

Reference List

Chapter 1 - General introduction


Chapter 1 - General introduction


61 Grant RT, Bland EF. Observations on arterio-venous anastomoses in human skin and in the bird's foot with special reference to the reaction to cold. Heart 1931; 15:385-411.


Chapter 1 - General introduction

Chapter 1 - General introduction


