



Microcirculatory assessment of vascular diseases

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Summary: The term “microcirculation” refers to the terminal vascular network of the body, which includes arterioles, capillaries, venules as well as initial lymphatic vessels. Additionally, it insinuates to their unique function in thermoregulation, fluid balance, maintenance of cellular exchange, and metabolism. Disturbances of microvascular function were identified to precede macrovascular involvement in the presence of cardiovascular risk factors and is the hallmark of terminal disease stages like critical limb or acral ischemia. Nevertheless, despite its obvious significance in vascular medicine assessment of microvascular function became increasingly neglected in the clinical institutions during the last decades and seems to play a subordinary role in medical education. We therefore provide an overview over relevant and clinically practicable methods to assess microcirculation in vascular medicine with critical estimations of their pros and cons and their perspectives in the future.

Keywords: Capillary microscopy, laser speckled contrast analyzer, O2C, tcpO2, indocyanine green fluorescence angiography

Introduction

Apparative, ultrasound-based, and radiologic methods support physicians to diagnose and stage vascular diseases [1–4]. In the past decades, pressure and ultrasound-based tools like ABI measurement dominated the primary diagnostic armamentarium of angiologists due to their non-invasiveness, delegability, and easy handling [5, 6], although there is clear evidence now that it is the microvascular function that is altered first in the presence of cardiovascular risk factors associated with atherosclerosis and that it is the condition of the microcirculation (and not pressure values) that determines prognosis in critical disease stages [7–11].

Nevertheless, assessment of microvascular function has become almost an orphan art in angiology reduced to measurement of transcutaneous oxygen pressure in critical limb ischemia or diabetic foot syndrome and capillary microscopy in Raynaud’s phenomenon, at best.

During the 2018 VAS European Vascular Days in Milano, Italy, an international VAS Microcirculatory Group of experts in this field was founded to re-sensitize vascular physicians for microcirculatory issues in clinical institutions, research, and education.

The following paper provides an overview over clinically relevant and practicable methods to assess microcirculation in clinical settings.

Methods of microvascular assessment

Capillary microscopy

Videocapillaroscopy (VCM) is a simple, non-invasive, and relatively inexpensive imaging technique that allows in vivo morphological assessment of capillaries and evaluation of nutritional blood flow [12, 13]. It is usually performed in the nailfolds of the fingers but might also be applied at the nailfolds of the toes, the conjunctivae, the buccal mucosa, or the skin surface [12–14].

Technical aspects

The best magnification to evaluate nailfold capillaries in detail is $\geq 200\times$. Lower magnifications (80–100 \times) are used for overview-images of the architecture of the capillary bed and assessment of capillary density, which varies

from 7–12 capillaries per mm in nailfolds of the fingers and toes in the first row to about 30 per mm on the dorsal surface of the forefoot, up to 70 per mm on the skin of the fingers and toes in healthy subjects [12–15].

VCM is obtained by pilling the tube of a conventional microscopy to a HD-videocamera connected to a computer system, which runs an imaging and analyzing software or by using a contact-probe equipped with 100 × and 200 × contact lenses connected to a computer with comparable software. Hand-held devices with USB-computer connections or blue-tooth connections to iPhones or iPods are also available, but often lack official certification for medical use.

Preliminaries

The patients should rest in a calm room with ambient temperature (20–23 °C) for a minimum of 15 minutes before starting the examination.

Methodology

Skin capillaries are usually arranged vertically to the skin surface, but in the nailfolds the capillaries are oriented horizontally. Therefore, only the apex of the capillary loops can be visualized in the skin, while in the nailfolds the afferent loop, the apex region, and the efferent loop of the capillaries as well as the venules are visualized [12–16].

In the skin, there are usually 1–3 capillary loops in each papilla. The tip of the capillaries is often seen as a dot or comma shaped structure [13, 14]. Atypical capillary formations like branching, bleedings, and reductions in capillary density are pathologic findings [13, 14]. In the nailfolds, the following morphologic and functional parameters are assessed: visibility, architecture, capillary density, morphology, diameter, and length of the capillary loops, extracapillary findings like bleedings, and capillary flow characteristics [12–16]. Normal capillaries in the nailfolds are hairpin shaped or tortuous, while a reduced density, bleedings, elongated, branched or dilated capillaries must be considered as single pathologic findings, that might also be seen occasionally in healthy volunteers [12, 14, 17, 18]. This underlines the necessity to assess and classify the whole capillary pattern in order to avoid false positive diagnostic results [12, 14, 18, 19]. Dilated capillaries > 50 µm in the apex region are referred to as “megacapillaries” and are not found in healthy individuals but are an important aspect of scleroderma pattern [12, 17, 18, 19–23].

Nailfold video-capillaroscopy (NVC) should best be performed in the fingers D 2–5 [12, 15, 16]. As the thumb and the index finger often exhibits significant hornification they might not be examined without any significant loss of information [12]. The best visibility is generally found in the 4th and 5th fingers of the non-dominant hand, where the skin shows a greater degree of transparency than in the dominant hand as well as in white collar works compared to mechanists [12]. The patient usually sits in an upright position in front of the capillaroscope resting the palm of the hand on its observation surface. Pressure on the skin or nails should be avoided, especially when

hand-held devices are used. With these devices the examination can also be performed in a supine position without further technical support, while a pivot arm is necessary for examinations in a supine position using a tube-microscope. To increase transparency, a drop of oil is placed on the nailfold bed of each finger or the skin region of interest. Fingers formerly affected by trauma or scarred regions of the skin should be excluded from analysis.

Clinical applications

While angiologists were once leading in this field during the last decades NVC has found much more attention amongst rheumatologists. Many new findings and re-definitions were published especially in patients with connective tissue disorders [12, 20–29]. VCM was promoted as a first line diagnostic tool in Raynaud’s phenomenon and in suspected connective tissue diseases, especially systemic sclerosis [12, 30, 31]. It proved helpful to differentiate between a normal capillary pattern (see Figure 1), which is easy to learn and reproduce, unspecific morphologic or functional changes, and specific capillaroscopic pattern associated with scleroderma spectrum disorders [12, 32–34].

The best described pathologic pattern in VCM is the “scleroderma pattern” (SP) found in systemic sclerosis (SSc) and associated connective tissue diseases. The SP is characterized by a disturbance of the capillary architecture, a diminished capillary density, the presence of enlarged capillaries and megacapillaries (giant capillaries), hemorrhages, and neo-angiogenesis [19, 20, 23]. In 1983, Maricq and Le Roy first described two main types of capillaroscopic patterns in scleroderma with prognostic implications. The “slow pattern”, which was characterized by ectatic capillaries and the absence of extensive avascular areas, was associated with a less severe disease manifestation, while the “active pattern” characterized by an extensive loss of capillaries and advanced architectural de-arrangement associated with more severe disease [35]. Cutolo et al. re-defined scleroderma pattern in early, active, and late pattern [20–23]. Figure 2 describes an example of an active pattern. Similar morphologic changes can be found in patients with dermatomyositis as well as in yet undifferentiated connective tissue disease, referred to as “scleroderma like pattern”, while systemic lupus erythematoses is not characterized by a pathognomonic capillary morphologic pattern [12, 24, 25, 28, 36, 37]. If antiphospholipid syndrome is present, it might be characterized by hemosiderin deposits arranged linearly around the capillary loops caused by microbleedings and/or blunt capillary thrombosis [38–40].

The risk of developing a digital ulcer in scleroderma patients is highly defined by the capillary density: The lower the density, the higher the risk of developing a new ulcer and the lower the probability for ulcer healing. This correlation is the main basis for the CSURI, a capillary morphologic algorithm defined as $DxM/N2$, where M is the maximum diameter of the megacapillaries, M the number of the megacapillaries and N the number of capillaries. At least one megacapillary must be present in the image selected [41, 42].



Figure 1. Normal appearance and density of capillaries in the human nailfold. Notice normal density and regular appearance of the capillaries in the first row.



Figure 2. Scleroderma active pattern (according to Cutolo et al. [20–22]). Notice apex enlargement, megacapillaries, irregular capillary width, bleeding formation, and edema.

Essential acrocyanosis, another frequent functional vascular acrosyndrome, is characterized by the prevalence of sometimes markedly enlarged capillaries and elongated tortuous efferent loops. This pattern is usually homogeneously distributed in all fingers and might also involve the sub-papillary venules [43].

In peripheral and acral arterial occlusive disease progressive capillary morphologic abnormalities and a loss of density of acral skin capillaries have been described [13, 14, 44]. According to Fagrell's classification stage A is characterized by dilatations and apical micropools, stage B by hazy appearance, edema, and capillary hemorrhages, and stage C by a progressive reduction of capillary density up to a complete loss of capillaries [13, 14, 44]. Fagrell's stages were found to correlate well with limb prognosis and can thus be used to assess amputation threat in critical limb or acral ischemia [13, 14, 44]. Almost the same findings were described by others assessing chronic venous disease, especially in CEAP stages 5 and 6, where capillary density and morphologic alterations are associated with impaired wound healing and ulcer (re-)occurrence [45, 46].

Despite clear prognostic implications, the assessment of skin capillary morphology and density in critical limb or acral ischemia as well as in chronic venous disease did

not prevail in clinical routine – probably due to the fact, that high quality handheld capillary microscopy devices making such an examination much more easy and reliable were not available until recently and skin alterations, hornification, and edema are limiting factors.

There are unspecific capillaroscopic findings associated with a large spectrum of rheumatological and non-rheumatological diseases ranging from diabetes and hypertension to orphan diseases like Fabry's disease and rare inherited disorders. In this large spectrum of disorders, capillary microscopy might be useful to discover microvascular dysfunction and capillary damage, yet the prognostic or therapeutic implications of these findings often remain unclear [47].

Future perspectives

The availability of hand-held capillary microscopes facilitates bed-site examinations or examinations in a supine patient's position. Thus, it is about time to re-evaluation the clinical usability and profit of VCM in critical limb or acral ischemia, and chronic venous disease with these new devices.

VCM might also become an important diagnostic tool for bed side assessment in critically ill patients. There is a growing number of studies being published in this area suggesting that the assessment of vascular density, blood flow heterogeneity, and – maybe most important – consequent early optimization of microcirculatory parameters may be associated with a better clinical outcome [48, 49].

There are first publications about automated analyzing software for VCM [48–50]. Artificial intelligence might thus be able not only to assist but also to automatically process and analyze capillary microscopy in the future [50–52].

Limitations

VCM examines morphologic and functional aspects of capillaries and venules. Larger vessels or thermoregulative blood flow is not in the focus of this method. Up-stream pathologies should be assessed prior to VCM.

Nailfold capillary microscopy as well as skin capillaroscopy is limited by hornification. As in ischemic conditions the degree of hornification increases, capillary microscopy in this condition is also often hampered by reduced transparency of the skin. Same is true for craftsman.

Examination of colored people is hampered by the increased pigment content of the skin and might even be impossible.

Quantification of capillary flow or flow velocity is difficult due to its heterogeneity and the need of additional equipment and software.

Laser speckled contrast analyzer

A laser Doppler is a non-invasive device to assess tissue microvascular perfusion. Depending on its penetration depth partial or total skin perfusion can be quantified

[53]. The technique is available in two different modes, the laser Doppler flowmetry and the laser Doppler imager resp. laser speckle contrast analyzer (LSCA) [54]. As Doppler flowmetry is nowadays mainly limited to scientific use only, we concentrate on LSCA in the description due to its wider use in clinical settings.

Laser speckle contrast analyzer

The LSCA reveals spatial heterogeneity of tissue or wound perfusion without direct skin contact [53–55]. The skin area of interest is scanned by a laser-beam (in the Perimed PeriCam PSI system a 785 nm-laser-beam is used, which is invisible for humans). The backscattered light is analyzed by a CCD-camera-system rendering up to 100 pictures per second [56].

Methodology

The technique is based on dynamic changes in the backscattered laser-light, which produces an interference pattern (speckle contrast pattern), that changes with changes in regional blood flow over time. Speckle contrast is defined as the ratio between the standard deviation of the intensity and the mean of the intensity. If there is high flow in the scanned tissue area, the standard deviation of the intensity will decrease and consequently the speckle contrast will decrease [54, 55]. The output signal is displayed in a color-coded image on a computer monitor: poor perfusion is usually expressed by blue, whereas higher perfusion is displayed by increasing quantiles of green, yellow and red [54–56]. A computer console is available for advanced analyses [56].

Preliminaries

The patients should rest in a calm room with ambient temperature (20–23 °C) for a minimum of 15 minutes before starting the examination. Convection and temperature changes have to be avoided.

Clinical applications

By means of this technique tissue perfusion in vascular acral syndromes and ulcers of various pathologies have been examined. Especially Cutolo and co-workers recently published a series of papers describing the usefulness of LSCA in the evaluation of Raynaud's phenomenon and scleroderma angiopathy [57–61].

According to these publications LSCA detected significant reductions of blood perfusion in areas usually affected by Raynaud's phenomenon (fingertips, periungual and palm areas), especially in patients with diffuse cutaneous systemic sclerosis. Perfusion values significantly correlated with the nailfold capillaroscopy scores of microangiopathy and showed progressive severity in early, active, or late stages [59]. Perfusion imaging with LSCA also correlated with skin dermal thickness at the level of dorsum of the middle phalanx of the third fingers in scleroderma patients [59]. LSCA also reliably monitored digital ulcer evolution and healing in systemic sclerosis, by evaluating areal skin

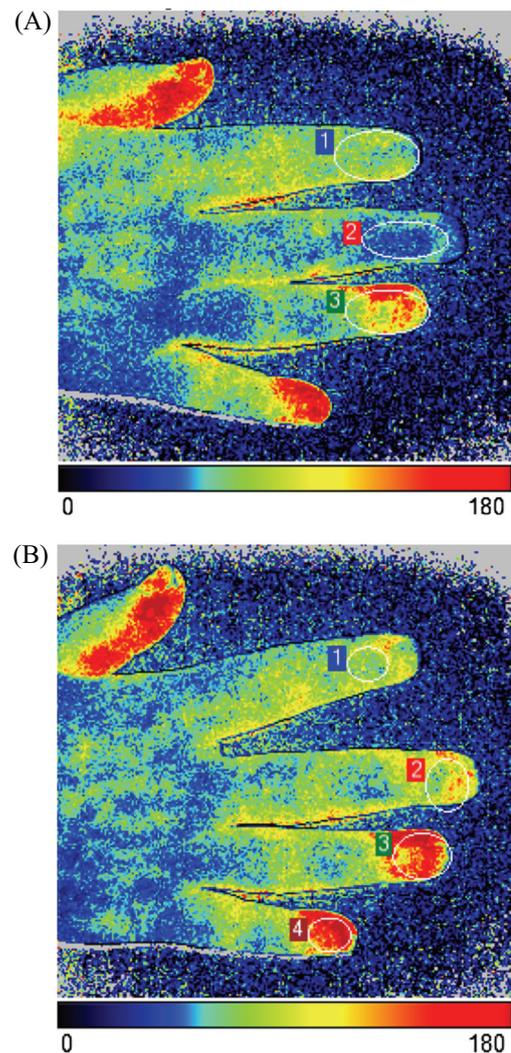


Figure 3. LSCA (Laser Speckle Contrast Analyzer)-Images. (A) Scleroderma digital ulcers II–III fingers. Notice malperfusion of the finger tips D III and III. (B) Same patient after Iloprost-infusions as well as nifedipine (long acting) and antiplatelet therapy p.o. Notice improved perfusion in the fingers, especially D II and III.

blood perfusion under standard treatment [61, 62]. An example is given in Figure 3.

The same group also published data about the inter-rater agreement and reliability of LSCA, which seemed to be good to excellent [62, 63].

In wounds, areas without granulation tissue usually shows low perfusion, while in granulation tissue perfusion is high indicating wound healing [64–66]. Scars usually show intermediary levels of perfusion. In intact skin adjacent to ischemic ulcers, perfusion is low reflecting overall ischemic conditions, while in chronic venous incompetence pathologic hyperperfusion of the skin or low perfusion (f.e. in atrophie blanche) might be found [67].

These findings led to the conclusion that wound healing is achieved by an increase of subpapillary perfusion in granulation tissue, while after formation of a scar it decreases to physiologic levels. In normal skin subpapillary perfusion is physiologically low, while in chronic venous incompetence it is often pathologically elevated due to hypertensive microangiopathy [64–67]. Additionally, an

increase of subpapillary perfusion was documented by laser Doppler imaging after the application of prostanoids [68].

Another clinical application of LSCA is burn-injuries, where laser Doppler systems assess burn depth with a good clinical correlation [69, 70].

Future perspectives

The advantages of LSCA are the examination of relatively large skin area instead of a punctual measurement enabling the examiner to detect areal perfusion heterogeneities and the clearness of its documentation. LSCA is an easy to perform diagnostic tool with multiple clinical applications reaching from functional vascular acrosyndromes to critical limb ischemia, monitoring of wound-healing, monitoring of medical interventions, and pre- and postinterventional or operative controls.

Limitations

The relatively small penetration depth only allows perfusion measurements of the upper skin areas. Stronger skin hornification, necrotic tissue, or scars limit its use. There are also motion artefacts in agitated patients or tremor, which might hamper or even preclude interpretation.

O2C (Oxygen-to-see™; micro-lightguide spectrophotometry)

O2C is a diagnostic device for non-invasive assessment of blood flow, blood content and oxygen supply in soft tissues by use of glass fiber probes. The method is also referred to as spectrophotometry or micro-lightguide spectrophotometry in the literature.

Technical aspects

A white light emitting probe also connected to a laser source radiates the skin and the underlying tissue. An integrated sensor detects the remitted light signals, while a computer determines doppler shifts by fast Fourier analysis. To determine the hemoglobin content and the oxygenated hemoglobin in the sample volume a wavelength range of 500–850 nm is used while flow is determined at 830 nm [3, 10].

O2C can be used as a single or multi-channel system with one channel recording the superficial tissue hemoglobin content and oxygen saturation of hemoglobin (e.g. of the skin), while a second channel monitors the oxygen supply and hemoglobin values of deeper tissues (e.g. subcutis, skeletal muscle) if a second probe with extended measurement volume is used. Maximal tissue penetration is about 2–3 cm.

- rHB reflects the hemoglobin amount in the tissue volume. As hemoglobin usually only circulates inside the (micro-)vessels this parameter reflects the filling of these vessels which itself depends on capillary density, capillary recruitment, and venous filling [3, 10]. The values are given in arbitrary units.
- SO₂ (ven). As more than two third of blood volume in the microcirculation is located in the venous pool with

this parameter mainly venolar oxygen saturation values are being measured. In contrast to the arterial oxygen saturation SO₂ (ven) reflects a balance between oxygen delivery and consumption. Low SO₂ ven-values indicate tissue hypoxia [3, 10]. Values are given in % of rHb.

- Flow is calculated by Doppler-shifts of emitted laser light induced by the movement of erythrocytes in the microcirculation [3, 10]. As the exact doppler angles in the tissue cannot be determined the values are given in arbitrary units.

All measurements can be performed easily by applying and fixing a small probe to the skin or wound surface with an adhesive film without pressure exertion and plugging in the connector. There is almost no time delay or equilibrium phase making measurements very fast and sensitive for chronologically or pharmacologically induced changes [3, 10]. Reproducibility is good, when comparable test conditions and exact repositioning are ensured.

Preliminaries

As described above in other microcirculatory measurement systems the patient should relax under ambient temperature conditions (20–23 °C) for at least 15 min before measuring.

Clinical applications

O2C was evaluated in critical limb ischemia, diabetic foot syndrome and for the prediction of early outcomes after balloon angioplasty and bypass surgery [71–73].

Maneuvers like elevation of extremities or measuring in dependent positions are valuable supplements, which increase diagnostic accuracy. For example, bringing the foot in an elevated position of 65 cm (corresponding to a hydrostatic pressure of 50 mmHg) is often used for the detection of critical limb or acral ischemia if flow decreases < 10 AU and SO₂ drops below 10% [3, 10]. On the other hand, venous insufficiency is characterized by a steep increase in rHb and a drop in SO₂ (ven) below 10% in depending position of the legs [3, 10]. O2C also helps to define the prognosis in wound healing if a wound mapping is performed and mean SO₂ (ven) is ≥ 30% and none of the SO₂-values undercut 15% [3, 10]. As was already described above in the laser Doppler flowmetry-section provocation maneuvers like determination of post-occlusive reactive hyperemia, endothelial dependent and independent vasodilation, or reactions after oxygen insufflation or drug administration can also be performed with the O2C-device.

Future perspectives

O2C meets many requirements for clinical use especially in critical acral or limb ischemia, diabetic foot syndrome, wound healing, and monitoring of therapeutic interventions. Advantageous is the short time requirement for the measurements and the combination of three parameters, which also allow conclusions on metabolic cell activity.

Combining this technology with high resolution ultrasound systems might be a wise further step to exactly

determine the tissue structures and to observe and measure regional tissue or organ perfusion in definite sectors.

Limitations

O2C is a punctual measurement, reflecting only a small tissue volume. For areal perfusion analysis a mapping with consecutive multiple measurements is necessary.

Penetration depth cannot be determined exactly in tissues. There are only theoretical values. Thus, the exact anatomical point of measurement actually remains unclear.

Transcutaneous oxygen tension (tcpO2)

Transcutaneous oxygen measurement is another non-invasive diagnostic method to analyze skin perfusion.

Technical aspects

Historically, the method was significantly influenced by Clark et al. in 1953, who developed a special electrode with a heating element, an oxygen permeable membrane and a measurement chamber containing a microanode and -cathode [74]. This basic construction principle still operates in modern devices. Alternatively, a photo-optical measuring system that does not consume oxygen while measuring has been developed and successfully launched on the market [75].

Under hyperthermic conditions of the skin the surrounding O₂ partial pressure is lower than the underlying arterial oxygen pressure. This pressure gradient varies depending not only on the arterial oxygen pressure and the local air pressure, but also on the temperature, the tissue oxygen consumption, the tissue blood-flow, and capillary O₂-shunting in the tissue [76]. Additional factors like the thickness of the epidermis, the degree of hornification, and contamination of the skin with ointments etc. also influence results. Heating a thermoelement over 43 °C induces local vasodilation underneath the probe, which is essential for facilitating O₂ diffusion, standardization, and reduction of intrinsic errors.

Using a Clark electrode-based system on the forefoot tcpO₂ values of 60–70 mmHg can be expected in a supine position [3]. On the other hand, values below 30 mmHg reflect critical limb ischemia by definition. Values below 10 have to be considered as being highly critical for the development of tissue necrosis respectively for non-healing of wounds and decubiti [3, 77–79]. The predictive value might be increased by performing provocation manoeuvres like measuring during leg dependency or oxygen inhalation [3, 77, 80].

Studies confirmed a hyperbole correlation between tcpO₂ and ankle-brachial index (ABI) [77]. TcpO₂ best reflects the degree of arterial perfusion deficit in the non-linear section of this hyperbolic function, i.e. at ABI values ≤ 0.5 [3, 77].

In the study by Urban et al. the absolute difference between the Radiometer TINA device and the Medicap device was approximately 15 mmHg in favor of the Medicap system [81]. Thus, tcpO₂ values can not be

compared directly between both methods, which indeed are based on different physiologic phenomena. Comparing regional perfusion indices (RPI, see below) might help to overcome these difficulties.

To increase validity, indices between different measuring points or additional provocation maneuvers were proposed:

1. Regional perfusion index is used to eliminate cardio-respiratory influences and simplify interpretation [80]. The reference electrode is placed on the chest.

$$RPI = \frac{tcpO_2 \text{ limb}}{tcpO_2 \text{ chest}}$$

Quotients of < 0.4 predict poor limb-associated outcome due to critical ischemia, while indices > 0.6 are usually associated with good limb associated prognosis and wound healing [82].

2. Measuring exercise transcutaneous oxygen pressures under standardized conditions, f.e. treadmill testing. This method has been used to confirm peripheral arterial disease in volunteers. A drop of 15 mmHg in the tcpO₂ from baseline values while walking was determined to represent a pathologic test result [81, 83].
3. Repetitive measurement in a depending limb position increases sensitivity of critical limb ischemia when an adequate increase in tcpO₂ is absent. The increase in depending position is called “microcirculatory reserve function” (MCR). MCR was shown to predict response to therapeutic interventions and limb outcome [78].
F.e., in spinal cord stimulation MCR (tcpO₂ in depending position – tcpO₂ in supine position) > 17 mmHg predicts good response to the procedure, while values < 17 mmHg predict poor limb outcome even in case of stimulation [84].
4. An increase in tcpO₂ under oxygen inhalation is also considered to be a sensitive measure of the degree and compensation of critical limb ischemia [80].

Preliminaries

For valid tcpO₂ measurements the patient should be placed in a supine position and remain in a stable ambient temperature of 21–23 °C after relaxing for at least 15 min. Smoking and coffee intake should be prohibited prior to the measurement. Clark electrodes have to be calibrated to local air pressure conditions prior to the measurement, while the photo-optical system has an automatic calibration function.

The skin under the probes should be prepared by removing hornification and cleaned with alcohol. Electrodes should be placed carefully at sites without scars, hairs, or skin defects as well as apart of skin veins. To avoid leaking adhesive pads are used for proper fixation.

Clinical applications

TcpO₂ measurement is used in low ABI values to improve prognostic accuracy and to predict wound or amputation healing.

TcpO₂ predicts short term outcome after below the knee angioplasty in critical limb ischemia [85] and wound healing in limb ischemia and in chronic venous incompetence [78, 79]. TcpO₂ has also been shown to predict amputation high in non-reconstructible peripheral arterial occlusive disease [86]. For this purpose, multiple measurements preferably with multichannel systems in different regions of the foot or lower limb are necessary (mapping, determination of isobares). Values < 10 mmHg in a supine position strongly argue against stump healing, while a cut-off value of 20 mmHg had a negative predictive value of about 50% [3]. Nevertheless, there are limitations like the presence of skin edema, local infections, scarring or thickening of the skin that do not allow accurate measurements. Furthermore, one has to bear in mind that tcpO₂ only reflects skin perfusion, not perfusion of deeper soft tissue structures like calf muscles.

Future perspectives

TcpO₂ is already well implemented in vascular medicine and there is a relatively wide distribution and a high level of experience with this method. Implementation of new provocation tests could extend effectivity and validity especially in the early stages f.e. of diabetic angiopathy where the sensitivity and specificity of standard tests like ABI is relatively low.

Limitations

TcpO₂-measurement with a Clark electrode is time consuming. Standard Clark electrodes need about 15–20 min to assure steady state measuring conditions. With the Medicap system time loss is much lower, which results in a high economic advantage in clinical routine, but both methods are not comparable by numbers, but at best by calculations of regional perfusion indices (RPI). Thus, all absolute values published for Clark electrode (f.e. 30 mmHg in the actual definition of critical limb ischemia) measurements must not be transferred for measurements with a photo-optical system.

TcpO₂ measurement is used in low ABI values to improve prognostic accuracy and to predict wound or amputation healing. Nevertheless, there are limitations like the presence of skin edema, local infections, scarring or thickening of the skin that do not allow accurate measurements. TcpO₂ reflects skin perfusion only, not perfusion of deeper soft tissue structures.

Indocyanine green fluorescence angiography

Fluorescence angiography represents an imaging method that uses the optical phenomenon of fluorescence. The technique itself is well known and has been applied for various indications as the assessment of microvascular perfusion especially in ophthalmology. More recently, the fluorescence angiography is getting more and more important with regard to wound healing and perfusion

assessment in CLI patients as well as in reconstructive surgery.

Technical aspects

The optical phenomenon of fluorescence is induced by electromagnetic radiation that is absorbed by fluorochrome substances in tissue that are thereby excited. These substances are visualized as excitation leads to emission of light. Particular substances carry an auto-fluorescence effect after irritation using specific wavelengths [87]. However, in vascular medicine a fluorochrome that increases contrast is of major relevance. Therefore, Indocyanine green (ICG) is widely used for vascular indications [88].

ICG is a water-soluble fluorochrome, which needs to be infused intravenously. ICG is transported in blood linked to plasma proteins and metabolized by the hepatobiliary system. Due to its properties, it can be used safely in patients with chronic kidney failure, which represents a relevant advantage in CLI patients. Iodine allergy poses a contraindication for ICG, though rates of further adverse reactions is very low (4:240.000) [89].

After intravenous application of the fluorescence dye, the ICG is excited by a near infrared light source and the tissue perfusion can thereby be visualized up to a depth of 5–7 mm. The absorption peak of ICG is at 800–810 nm, the emission peak at 830 nm [90].

There are several fluorescence angiography systems for the assessment of tissue perfusion commercially available.

An interpretation and quantification of the video sequences can be achieved on base of a grey scale. The different fluorescence intensities are encoded in a 256 grey scale matrix. Different fluorescence systems are available with already integrated analysis software and tools. Based on a grey scale score, the video sequences are analyzed. Different relative parameters are defined for quantification, such as Ingress (IN, difference of the maximum – minimum intensity in each sequence) and the Ingressrate (InR, increase of fluorescence intensity per second). Other analysis tools aim to quantify the time to accumulation (time to slope, i.e. the time from ICG application to first detection in tissue).

Preliminaries

Measurements should be performed in a stable ambient temperature of 21–23 °C after relaxing for at least 15 min. As an intravenous application of ICG is necessary and allergic reactions have been reported, written consent should be secured.

Clinical applications

The first clinical trials with ICG in humans were performed in 1957 at the Mayo Clinic, the US Food and Drug Administration approved the method already in 1959 [91]. Initially, fluorescence angiography was clinically used in intensive care medicine for measurement of the liver function by the so called ICG clearance test [91]. In the field of cardiology, ICG was then used to monitor cardiac

output and to visualize outflow of single cardiac bypasses intraoperatively [92]. Its most widespread application is nowadays in ophthalmology, where fluorescence angiography was implemented in the 1970s to visualize retinal perfusion [93]. In recent years, ICG found wide acceptance in the fields of plastic and reconstructive surgery to monitor free tissue transplantations, as well as in the field of vascular and transplantation surgery [94–102].

A significant advantage of the ICG angiography is its relatively simple intraoperative application compared to other angiography methods (x-ray, CTA, MRA). Intraoperatively, ICG can be used for direct visualization of the bypass graft and the anastomosis for patency control. Another application is the assessment of tissue perfusion on the level of microcirculation. This aspect is of special interest in patients suffering from chronic critical limb ischemia (CLI) [99, 101].

Several studies have been performed to implement the fluorescence angiography technique in vascular procedures [96, 98, 100, 101]. After tibial bypass surgery this method can be used to detect an improvement of the foot microcirculation, that additionally correlates with the macrocirculation (ankle-brachial-index) [101]. The reproducibility has been tested and revealed a high level of concordance in sequential measurements [100]. Significant correlation to other methods of perfusion analysis such as tcpO₂ has been shown as well [100].

Compared to other perfusion assessments, the advantage of the fluorescence angiography investigation is the visualization of tissue perfusion. This gives the investigator an immediate impression of the foot's regional perfusion or perfusion deficits. An example is given in Figure 4. On base of these findings, minor amputation procedures can be planned, and the perfusion of wound beds can be investigated.

Another interesting application of ICG fluorescence angiography is its use in kidney transplantation surgery [94, 95, 102, 103]. As common digital subtraction angiography is not applicable due to its potential nephrotoxic effects, there is a need of an alternative intraoperative quality control. ICG fluorescence angiography represents a new alternative option, that has recently been established. In addition to the visual intraoperative organ perfusion control, the fluorescence parameters Ingress and Ingressrate have been established for quantification. These two parameters can predict an impaired organ function at the time of transplantation, as pre-existing chronic morphological changes in the kidney biopsy specimens lead to significant reduced perfusion parameters intraoperatively [102].

Future perspective

As there are multiple areas of application and the use is relatively simple, fluorescence angiography may gain more impact in the future for the investigation of skin or organ microcirculation. Further studies might focus on the inter-individual comparability of the fluorescence parameters



Figure 4. ICG fluorescence angiography of the forefoot. Notice ulceration and severe malperfusion of the first toe of the left foot in critical limb ischemia.

and to establish cut off values, which can for example predict clinical endpoints like wound healing.

Limitations

One main limitation of this method is its application in infected areas. In these patients, the perfusion analysis yields hyperperfusion that is incoherent with the real perfusion due to a reactive hyperaemia based on the infection.

While the dye is relatively inexpensive, the costs of the equipment is still very high. Additionally, potential allergic reactions have to be taken into account.

Discussion

Marcello Malpighi in 1661 first discovered tiny vessels linking the arterial and venous system and thus introduced the concept of microcirculation [104]. Soon after his observation Antoni van Leeuwenhoek described the movement of blood cells in frog capillaries with a self-made microscope [105] and Johan Christophorus Kolhaus in 1663 noticed similar small vessels around the nails of humans [104], an observation that lay the foundation of modern nailfold capillaroscopy [106].

Since these early description's microcirculation gained variable attention by scientists and physicians over time. Periods of high appreciation and high scientific and technical output alternated with phases of negligence sometimes without plausible reasons.

Assessment of microcirculation became a cornerstone and a driving force in what could be retrospectively called "the golden age of angiology", when the scientific and clinical fundamentals of the discipline were built (period between the 60th and 90th of the last century). What made angiologists successful at that time was a close connection between fundamental research especially in microcirculation and clinical research with high volume output and consecutively high scientific reputation of its representatives. In contrast, a significant output in microcirculation research from angiologists is nowadays lacking, while

rheumatologist discovered microcirculation as a new field of interest already dominating clinical publications in by far.

The reasons for this development might be numerous. First of all, the spread of ultrasonographic vascular diagnostics and developments in ultrasonographic devices with increasing resolution that have made the study of the macrocirculation much more easy, fascinating, and profitable for angiologists than sitting in front of a capillary microscopy or other time-consuming devices, which are poorly refunded or subsumed in an all-inclusive refunding system with obligatory duplex provision. Additionally, angiologists became increasingly engaged in vascular interventions in many countries, concentrating more on interventional techniques and progress in this field. Furthermore, in former times there were much more common scientific activities and meetings between vascular biologists and vascular clinicians, which led to synergistic effects and common scientific activities, while such points of contact and exchange are widely missing today.

In a long run this development might take revenge and uncouple angiologists from potentially rewarding future developments in medicine as new devices for microvascular assessment appeal with rewarding perspectives by coupling morphologic and functional data on the vessel-cell and metabolic interface.

Already it became clear, that the development of cardiovascular complications in metabolic diseases like diabetes mellitus, obesity, hypertension, and hyperlipidemia is mainly driven by disturbances of microvascular disturbances which are already prevalent at time-points where macrovascular involvement is still absent [8, 9, 107–113].

This sequence opens new opportunities for an earlier identification of patients at risk and intensified primary prevention. The authors are also convinced, that on the other end of the spectrum clinically relevant therapeutic progress in yet deleterious conditions like non-reconstructible peripheral arterial or severe acral occlusive disease will only be possible by increasing the input in microcirculatory research, clinical microcirculatory assessment, and surveillance and solving problems in standardization, and definition of clear cut-off values.

The authors propose to reinforce knowledge and expertise in the field of microcirculation amongst vascular medical students and specialists and to upgrade microvascular assessment and disturbances in the curricula of vascular medical specialization in Europe.

As was shown in our overview, methods to assess different aspects of microvascular dysfunction have increased and already provide new and exciting fields of scientific and clinical applications inducing an urgent need for better standardization and clinical evaluation.

New developments coupling (micro-)imaging with functional data and artificial intelligence are under way, which will even expand our possibilities and the informational value and content of microcirculatory assessment in the future.

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History

Submitted: 29.09.2019

Accepted after revision: 29.12.2019

Published online: 10.02.2020

Conflicts of interests

No conflicts of interest exist.

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