MICROCIRCULATION: ASSESSMENT TECHNIQUES*

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ABSTRACT

While intravital microscopy of the microcirculation in the transparent tail of small fish has been popular as early as in the 18th century, only recently a broader array of techniques to investigate microcirculatory parameters in different organs in patients has been developed. The scope of microscopical techniques was expanded by the introduction of instruments based on orthogonal polarization and laser scanning ophtalmoscopes. They allow the measurement of morphological (diameter, length) and functional (flow velocity) parameters for individual microvessels. Also, parameters describing characteristics of small regions of terminal vascular beds (e.g., microvascular density) can be evaluated. The other group of techniques to assess mcrocirculatory function and abnormalities uses integrating approaches. On skin or organ surfaces local flow can estimated by laser doppler probes and imagers while similar measurements for whole organs may be made using catheter techniques and plethysmography. The newest, very powerful additions to the methodological arsenal are the imaging techniques of positron emission tomography (PET), magnetic resonance imaging (MRI) and contrast echocardiography (CE). They allow non-invasive measurement of regional blood flow in internal organs, e.g. the heart. Since base line values obtained with any of the above methods are difficult to interpret due to the large intra-and inter-individual variability of microcirculatory parameters, the information content of such measurements is often increased by using provocation tests. Such maneuvers include exercise, arterial occlusion/reactive hyperaemia, application of vasoactive agents or in case of the skin, local heating. These new techniques increasingly allow the assessment of microcirculatory abnormalities from early events such as endothelial dysfunction to established end organ damage.

The microcirculatory section of vascular beds consists of arterioles, capillaries and venules with diameters below 100-300 μm. This part of the vasculature has eminent functional importance in controlling blood flow distribution and peripheral resistance, exchange with the tissue and immune reactions involving extravasation of leukocytes. The vast majority (~ 98%) of the total endothelial

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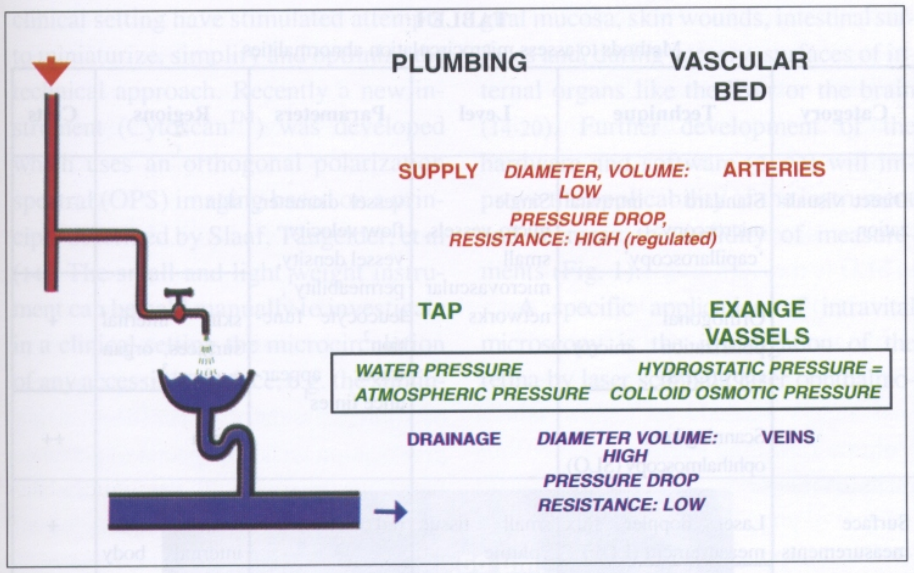

surface of about 300m^2 is located in the microcirculation. Thus, microvascular abnormalities play a central role in a number of pathologies, notably in hypertension. It has, however, not been easy to investigate microcirculatory structure and function non-invasively in patients. For a long time, the main approach was intravital microscopy of the skin (and nailfold). This technique provides access to a very limited range of vessels which are not necessarily representative for the pathology studied. In addition, the large heterogeneity of all pertinent parameters for microvessels (1) renders the interpretation of obtained data very difficult.

During the last two decades, a substantial number of new developments and approaches ranging from improvements of intravital microscopy to sophisticated imaging technologies like positron emission tomography (PET) have widened the options for microcirculatory measurements in patients (Table I). These techniques broaden the range of tissues and functions accessible for investigation and allow measurement of functional parameters relating to the microcirculation in internal organs, e.g. the heart. For hypertension research, the clinical investigation of primary and secondary microvascular alterations as well as the effect of treatment modalities on such alterations bears a lot of potential.

Direct visualization of the microcirculation

Visualization of individual microvessels of the skin, the conjunctiva or tissue surfaces exposed during surgery is possible by direct microscopic investigation (intravital microscopy). During the last twenty years, the development of new optics, new visualization and measurement methods and new recording and analysis techniques substantially increased the applicability of intravital microscopy as a precise and versatile scientific tool. These improvements led to higher precision of measurements as well as to a larger range of phenomena which can be observed and quantified and intravital microscopy of the skin has successfully be used, e.g., for the analysis of microvascular alterations in hypertension (2,3).

TABLE I
Methods to assess microcirculation abnormalities

Category	Technique	Level	Parameters	Regions	Costs
Direct visualization	Standard intravital microscopy, 'capillaroscopy'	Single micro-vessels, small microvascular networks	vessel diameter, flow velocity, vessel density, permeability[1], leucocyte function[2], tracer appearance times[1]	skin	+
	Orthogonal polarization microscopy (OPS)			skin, internal surfaces, organ surfaces[3]	+
	Scanning laser ophthalmoscopy (SLO)			retina	++
Surface measurements	Laser doppler flux measurement (LDF)	small tissue volume elements	red cell flux	skin, internal body surfaces, organ surfaces[3]	+
	Reflectance spectroscopy, Transcutaneous PO_2 (TC-PO_2)		oxygen saturation, oxygen partial pressure PO_2		+
3D Imaging	Photon emission tomography (PET)	tissue volume elements	local blood flow or metabolic state	organs	++++
	Magnetic resonance imaging (MRI)		local blood flow		+++
	Contrast echocardiography (CE)		local blood flow		++
Regional measurements	Catheter techniques	organs, tissue regions	regional blood flow	organs, tissue regions	++
	Plethysmography				+

[1] Using tracers, e.g. fluorescent labels
[2] With special contrast enhancement, e.g. blue filtering
[3] During surgical procedures

The classical investigation of small capillary type vessels in the skin and the nailfold is called capillaroscopy (4-6). The use of digital image analysis allows a quantification of structural parameters (e.g. vessel diameter, length, shape) the local hemodynamics (e.g. flow velocity) and functional properties (e.g. permeability) (7-11). Specialized techniques may be used to investigate further parameters, e.g. micropipettes are used to measure capillary pressure (12), or fluorescent tracers allow measurement of appearance times, permeability or lymph vessel function. A further improvement in depth of penetration and resolution may be achieved by the use of confocal scanning laser microscopy (13).

The complexity of the instruments employed for standard capillaroscopy and the limitation in the range of areas and tissues which can be studied in the

clinical setting have stimulated attempts to miniaturize, simplify and optimize the technical approach. Recently a new instrument (Cytoscan™) was developed which uses an orthogonal polarization spectral (OPS) imaging based on a principle described by Slaaf, Tangelder, et al (14). The small and light weight instrument can be held manually to investigate in a clinical setting the microcirculation of any accessible surface, e.g. the sublingual mucosa, skin wounds, intestinal surfaces and, during surgery, surfaces of internal organs like the liver or the brain (14-20). Further development of the hardware and software (21,22) will improve the applicability of the instrument and increase the validity of measurements (Fig. 1).

A specific application of intravital microscopy is the investigation of the retina by laser scanning laser ophthalmo-

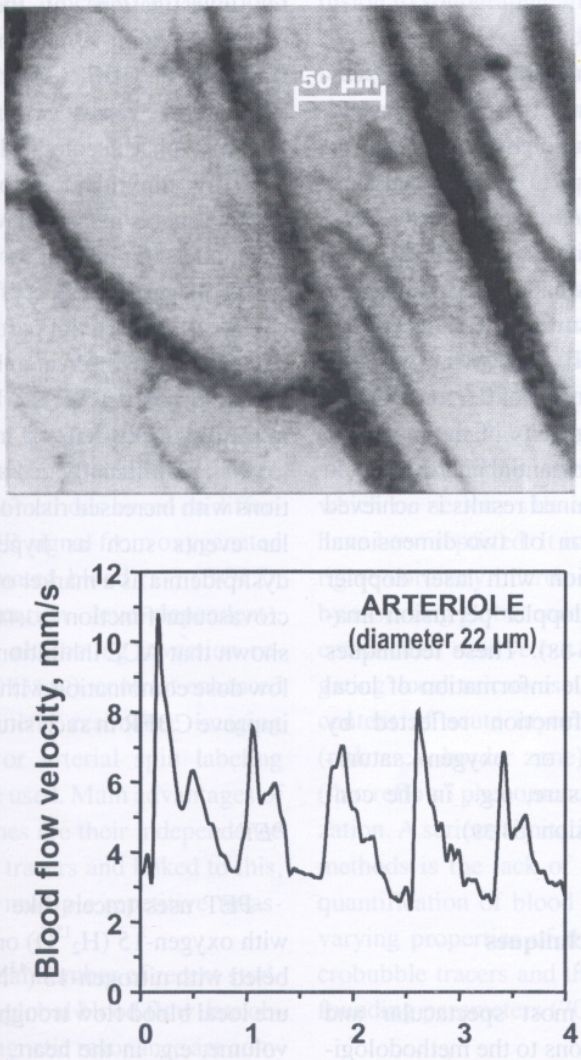

Fig. 1– Upper panel: Microvessels in the sublingual mucosa of a health subject as seen with the Cytoscan™ intravital microscope using orthogonal polarization spectral imaging (OPS). The granular structure inside the vessels relates to individual red cells 'stopped' in motion due to short flash exposure (21).

scopy (SLO) (23,24). This technique is of great interest because microcirculatory disorders are important for end organ damage in the eye and because the retinal circulation bears strong resemblance to brain circulation. Furthermore, the use of fluorescent tracers has been established in SLO to measure arterio venous transit times (25) and to analyze leucocyte function in vivo (26,27).

Volume sampling surface measurements

Laser doppler flow metry (LDF) (28-31) and a number of techniques to measure oxygen saturation or PO_2, like reflectance spectroscopy (32,33) and transcutaneous PO_2 measurement (TC-PO_2) (34,35), assess microvascular function by investigation of small volume samples of body and organ surfaces, e.g. the skin. In the case of LDF, the typical sampling volume is in the order of 0.5 mm^3. Due to the local heterogeneity of the measured parameters, a substantial improvement in the value of obtained results is achieved by the generation of two-dimensional maps of perfusion with laser doppler imagers (laser doppler perfusion imaging, LDPI) (36-38). These techniques can give valuable information of local microvascular function reflected by local perfusion or oxygen saturation/partial pressure, e.g., in the context of hypertension (34,39).

3 D Imaging techniques

Among the most spectacular and promising additions to the methodological modalities to investigate microcirculatory phenomena in a clinical setting are the imaging techniques positron emission tomography (PET), magnetic resonance imaging (MRI) and contrast echocardiography (CE). These techniques are non-invasive and provide access to internal organs like the heart, the brain or the kidney.

Due to the restricted resolution of these imaging techniques, they do not allow a direct investigation of individual microvessels. However, the possibility to measure global or local blood flow repetitively allows an indirect assessment of microcirculatory function using provocation tests. Of special importance for investigations of the myocardial microcirculation is the coronary blood flow reserve (CBFR), i.e. the quotient of blood flow values at rest and after maximal pharmacological vasodilation (e.g. by dipyridamole or adenosine) which can be measured with all three techniques. For normal coronary arteries, the CBFR mainly reflects flow resistance changes in the arterial part of the microcirculation (40-42). A number of studies have shown that CBFR which averages in the range of 3.5 to 4.2 in healthy subjects is significantly reduced in conditions with increased risk for cardiovascular events such as hypertension and dyslipidemia as a marker of impaired microvascular function (43,44). It was also shown that ACE inhibition alone or in a low dose combination with a diuretic can improve CBFR in such situations (45-47).

PET

PET uses tracers like water labeled with oxygen-15 ($H_2{}^{15}O$) or ammonia labeled with nitrogen-13 ($^{13}NH_3$) to measure local blood flow trough a given tissue volume, e.g. in the heart, the kidney or the brain, in absolute terms (e.g. in ml blood per tissue volume and time) (48-50). The dual positron emission of such tracers allows the use of coincidence de-

tection to correct for errors introduced, e.g., by local differences in attenuation.

An increasing number of studies shows that blood flow measurements by PET are valid and reproducible (51-55). Due to the short half life of the tracers ($H_2{}^{15}O$: ~2 min, $^{13}NH_3$: ~10 min) the indicator has to be prepared in the close vicinity of the tomography instrument, e.g. by specialized cyclotron systems. On the other hand, the short half life also allows repeated measurements with a short time delay which are crucial to estimate microcirculatory function. In addition, the use of specific tracers an protocols allows mapping of the metabolic tissue status or receptor densities.

MRI

Modalities based on magnetic resonance imaging have been recently developed to allow estimation of global [56] or local levels of blood flow. For the estimation of local oxygen consumption or blood flow with functional MRI, a number of different methods based on differences in the MR signal from oxygenated and desoxygenated blood (57) (BOLD, **b**lood **o**xygenation **l**evel-**d**ependent), **f**low **s**ensitive **a**lternating **i**nversion **r**ecovery (FAIR) (58,59), contrast-enhanced **d**ynamic **m**agnetic **r**esonance **i**maging (dMRI) (60) or **a**rterial **s**pin **l**abeling (ASL) (61) are used. Main advantages of MRI approaches are their independence of radioactive tracers and linked to this, the option of multiple repetitive measurements.

A substantial number of recent studies shows that global blood flow data obtained with magnetic resonance are comparable to those obtained with PET (62-65) or invasive techniques, e.g. a doppler wire (66). The true spatial resolution of MRI can be increased above that attainable with PET, however, the conversion of signals into absolute values, e.g., of tissue blood flow is a problem and may only be achieved for some modalities (ASL).

CE

The introduction of microbubbles filled with air or with gases of higher molecular weight as contrast agents has added the possibility to assess tissue perfusion to sonographic methods, or myocardial contrast echocardiography (MCE) if used on the heart. Reduction of bubble size and of gas diffusion from the bubbles increased bubble half life and allow venous application and lung passage of the tracers. Regional blood flow can be assessed by comparing contrast intensities with baseline values determined before injection of the contrast medium or after destruction of the microbubbles present in the tissue by a single, high energy ultrasound impulse (67).

MCE is a noninvasive technique for which no serious side-effects and risks have been reported. It is suited to investigate coronary microcirculation (68,69) by assessing global coronary or local microvascular reserve but also by investigating local microvascular flow in the context of acute myocardial infarction (risk area, border zone), recanalization ("no reflow" phenomenon) or collateralization. A serious limitation of the present methods is the lack of a true, absolute quantification of blood flow due to the varying properties of the available microbubble tracers and the effect of confounding parameters (70).

Regional measurements

Invasive catheter techniques can be

used for an investigation of specific functional properties of the microvasculature in tissue regions downstream of the catheter tip. Vasoactive substances are infused through an arterial catheter while measuring blood flow with a doppler wire or with the imaging techniques described above. By comparing flow values at rest and after maximal dilatation, the flow reserve of the entire tissue region supplied by the respective artery (doppler wire, PET, MRI) or the local microvascular reserve in visualized tissue volume elements can be analyzed (PET, CE). Furthermore, the state of endothelial function in the investigated tissue regions can be analyzed by successive application of vasodilator substances which act via the endothelium (e.g. acetylcholine) or endothelium independent by direct effects on the smooth muscle (e.g. adenosine) (71).

An indirect but also non-invasive approach to estimate endothelial function of the microvascular bed of the forearm is based on measuring blood flow with plethysmography (72). In this context procedures like, infusion of vasoactive agents (73) or reactive hyperaemia following complete flow stop with a cuff inflated to super-systolic pressures are used to assess the endothelial dilatory capacity.

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