Cardiovascular Research (2008) **80**, 165–174
doi:10.1093/cvr/cvn136

Review

A review of methods for assessment of coronary microvascular disease in both clinical and experimental settings

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Received 7 January 2008; revised 20 May 2008; accepted 23 May 2008; online publish-ahead-of-print 29 May 2008

Time for primary review: 23 days

KEYWORDS
Coronary microvascular disease;
Microvascular angina;
Plethysmography;
Laser Doppler flux;
Iontophoresis;
Intravital microscopy

Obstructive disease of the large coronary arteries is the prominent cause for angina pectoris. However, angina may also occur in the absence of significant coronary atherosclerosis or coronary artery spasm, especially in women. Myocardial ischaemia in these patients is often associated with abnormalities of the coronary microcirculation and may thus represent a manifestation of coronary microvascular disease (CMD). Elucidation of the role of the microvasculature in the genesis of myocardial ischaemia and cardiac damage—in the presence or absence of obstructive coronary atherosclerosis—will certainly result in more rational diagnostic and therapeutic interventions for patients with ischaemic heart disease. Specifically targeted research based on improved assessment modalities is needed to improve the diagnosis of CMD and to translate current molecular, cellular, and physiological knowledge into new therapeutic options.

1. Introduction

Ischaemic heart disease may frequently occur in the absence of significant coronary atherosclerosis, especially in women.[1–3] Clinical reports suggest that in these patients, chest pain is related to coronary microvascular disease (CMD).[4] CMD has been suggested to be present also in many other patients after relief of significant coronary atherosclerosis. Cardiologists are familiar with the phenomenon of slow myocardial blood flow in the setting of acute myocardial infarction[5] or with the occurrence of persisting chest pain, and ischaemia, after percutaneous coronary intervention in the absence of residual stenosis in the culprit vessel.[6]

The implicit assumption in all of these patients is that reduced perfusion in the absence of significant stenosis would reflect impairment of the myocardial microcirculation. This assumption however needs confirmation.

It is the aim of the present article to briefly review the current knowledge on the involvement of CMD in different clinical scenarios and to define diagnostic approaches that are currently available or under development to assess CMD both invasively and non-invasively. This article has been prepared by members of the Working Group on Coronary Pathophysiology and Microcirculation of the European Society of Cardiology (ESC) and reflects their expertise and knowledge of this field.

2. Endothelial function/dysfunction

Clinical symptoms of patients with non-obstructive coronary disease are often poorly understood. Chest pain could be explained by microvascular dysfunction. Abnormality of both endothelium-dependent and -independent vasodilatation

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may be a cause of vascular dysfunction, the most frequent cause being endothelial dysfunction.[7–9]

A normally functioning vascular endothelium is required for appropriate dilatation of arteries. Endothelial cells produce several mediators with vasorelaxing, anti-proliferative, anti-thrombotic, and anti-adherent effects, such as nitric oxide, prostacyclin, endothelium-derived hyperpolarizing factor, and C-type natriuretic peptide. These factors are balanced by the release of substances with opposing effects, such as endothelin, thromboxane A_2, prostaglandin H_2, and superoxide anion. Impairment of endothelium-dependent dilatation shifts a net dilator response to a variety of stimuli to a net constrictor response.

3. Coronary microvascular disease in different clinical scenarios

There are three broad categories related to possible abnormalities of the coronary microcirculation. The first is referred to as the occurrence of ischaemic heart disease in the absence of angiographically significant coronary atherosclerosis and could result from inflammation and/or abnormal vasomotor regulation via endothelial-dependent and independent pathways.[1–4] However, it should be considered that a normal or near-normal angiography does not necessarily rule out the presence of a large 'hidden' atherosclerotic burden.[10,11] The second category refers to inadequate post-PCI and/or post-thrombolysis coronary reperfusion entailing an early phase referred to as 'microvascular obstruction'[12] possibly involving microembolic mechanisms, and a later phase of 'microvascular dysfunction', which may represent corollaries of reperfusion injury, including tissue oedema, neutrophil aggregation, and free-radical release.[13,14] Finally, the third category is microvascular dysfunction in the context of epicardial vessel disease.

3.1 Acute coronary syndrome in the absence of significant coronary atherosclerosis

This phenomenon has been considered for many years as an angiographic curiosity. New data suggest that this is no longer appropriate. Patients with chest pain and normal or near-normal coronary angiograms belong to a group in which the prognosis is not necessarily as benign as previously reported[2] especially if impaired endothelial function is diagnosed.[15,16] Recent work revealed that the 1 year mortality rate of patients with acute coronary syndrome (ACS) but without obstructive coronary artery disease is high (1.0%), but these patients have a relatively lower incidence of death compared with patients with obstructive coronary artery disease in the same clinical setting (3.9%).[17] The death rate in patients with chronic stable symptoms ranges from 0.6% per year in the absence of obstructive coronary lesions to 2.0% in the presence of such lesions.[18,19] Patients with ACS are, therefore, usually at higher risk than patients with stable effort-related angina even in the absence of coronary obstructions. This demonstrates that we need more information on mechanisms underlying myocardial ischaemia and cardiac damage in patients presenting with an ACS and non-obstructive epicardial coronary disease. We also need reliable methods of risk stratification of patients with non-obstructive coronary disease.[20]

3.2 Chronic stable angina in patients with angiographically smooth normal coronary arteries

Recurrent chronic stable angina in patients with angiographically smooth coronary arteries and an abnormal stress test response has been referred to as 'Cardiac Syndrome X'.[1,21,22] In a subset of patients with Syndrome X, microvascular dysfunction can be demonstrated and this entity is commonly referred to as 'microvascular angina'.[23]

Microcirculatory dysfunction has recently been associated with heart failure and/or diabetes and hypertension.[4,5,24] Early-stage atherosclerosis is also associated with endothelial dysfunction and may contribute to microvascular dysfunction in less defined clinical subsets of patients.[23,25,26] Finally, microvascular vasomotor dysfunction may occur independently of epicardial disease in heart transplant recipients, suggesting different entities of immune-mediated cardiac allograft vasculopathy,[27] which may have prognostic importance for the deterioration of left ventricular function.[28] A better understanding of the mechanisms of recurrent chronic angina in the absence of coronary disease at angiography should allow assigning an increasing fraction of these patients to distinct pathophysiological entities, which, in turn, may support the development of more specific treatment options.

3.3 Persistent/recurrent angina after successful revascularization

In many cases, percutaneous or surgical revascularization results in superior symptomatic relief of angina and improves exercise tolerance compared with medical therapy, but the benefits are seldom complete. It is common clinical experience to see that a substantial portion of patients still continues to experience symptoms under various circumstances. The long-term effects of PCI in comparison with an alternative path of continued medical treatment have been reviewed recently.[6,29] At 5 years, 26% of patients in the PCI group and 28% of those in the medical therapy group showed persisting angina. Similar observations were reported in a multinational prospective study comparing the relative benefits of CABG and PCI in patients with multivessel disease potentially amenable to stent implantation.[30] Although several mechanisms may be considered to explain the persistence of angina after a revascularization procedure (incomplete revascularization, graft/PTCA failure, and disease progression in native coronary arteries), the unexpected prevalence of angina after successful revascularization supports the hypothesis that persisting alterations of microcirculation contribute to the pathogenesis of ischaemia.[31,32]

3.4 No reflow

In patients with acute myocardial infarction, inadequate myocardial perfusion after successful coronary recanalization (no reflow) is associated with adverse cardiovascular events.[33,34] No reflow may be associated with lack of patency or with loss of anatomic integrity of microvessels. The former is potentially reversible, whereas the latter is associated with definitive tissue damage.[5]

Multiple mechanisms may account for this finding. Necropsy studies have demonstrated the presence of thrombi in the coronary microvasculature from patients who died of acute myocardial infarction.[35] Multiple

mechanisms may account for this finding. Fibrinolysis generates elevated levels of free thrombin.[35–37] The release of vasoactive mediators from activated thrombocytes may impair regional flow (microvascular spasm) and contribute to the no-reflow phenomenon.[36,37] Oxygen free radicals generated soon after the release of the epicardial obstruction may inactivate nitric oxide, thus impairing endothelium-mediated vasodilatation, or directly cause microvascular spasm.[38] Flow may also be impaired by adhesion of leukocytes to microvascular endothelium.[39] Inadequate myocardial perfusion after successful coronary recanalization is associated with adverse cardiovascular events.[33,34] No reflow may be associated with lack of patency or with loss of anatomic integrity of microvessels. The former is potentially reversible, whereas the latter is associated with definitive tissue damage.[5] Although GP IIb/IIIa glycoprotein receptor inhibition generally ameliorates deficits in coronary flow reserve after acute myocardial infarction treated with primary PCI[40] and improves prognosis in these patients, a potent therapeutic strategy to prevent or improve the no-reflow phenomenon is still missing.

3.5 Microvascular dysfunction in the context of epicardial vessel disease

Coronary artery stenosis decreases flow reserve in the vascular bed distally to a stenotic epicardial artery.[41] Even early atherosclerotic lesions are associated with impaired endothelial coronary blood flow regulation.[42] In addition to the effects of a stenosis on flow reserve within its own bed, coronary stenosis and occlusion also decrease flow reserve in adjacent non-stenotic beds in both experimental animal models and clinical populations.[43] Importantly, global coronary flow reserve predicts cardiovascular events in patients with only minimal[44] or intermediate epicardial lesions.[45] It also predicts development of restenosis after coronary stent implantation.[46] Blood flow-mediated shear stress on the endothelium of epicardial vessels controls the release of nitric oxide and other factors, regulating the vascular milieu and therefore may interfere with progression of atherosclerosis or restenosis development.[47] The integrity of blood flow regulation in the microcirculation may thus have potential effects on the development of epicardial atherosclerosis.

4. Experimental assessment of coronary microcirculation

Investigating alterations of microcirculatory function under a variety of pathophysiological conditions is a very active area of research, which has much contributed to our understanding of microcirculation. Many experimental models are available to investigate the coronary microcirculation; each has its own peculiarities, strengths, field of application, and limitations.

Rats, pigs, and dogs have been used most frequently as models in the study of intramyocardial circulation to validate imaging techniques, to examine postmortem pathology, to elucidate the pathophysiology of microvascular tone, and to study the effectiveness of pharmacological interventions in promoting microvascular dilation. Experimental models to study microvascular diameters and/or perfusion as well as cellular and molecular mechanisms include (i) *in situ* measurements; (ii) isolated heart preparations; (iii) isolated vessel approaches; (iv) cultured coronary microvascular endothelial and smooth muscle cells. Models for intravital microscopy of the beating heart in open-chest animals have been published more than 30 years ago.[48,49] Later, microvascular diameter measurements by intravital microscopy in conjunction with luminal pressure determination have been performed to analyse the resistance distribution within the coronary microvasculature at rest and during dipyridamole-induced vasodilation.[50] Also, different longitudinal gradients in the responsiveness of coronary arterioles to adenosine and nitroglycerin,[51] and in alpha-1 and -2 receptor-mediated coronary constriction,[52] have been identified. A needle-probe microscope[53] allowed to identify differences between responses in epicardial vs. endocardial arterioles.[54]

4.1 *In situ* measurements

Perfusion *in vivo* may be measured invasively with intracoronary Doppler wires or with flow sensors positioned around the vessel in open-chest preparation or non-invasively with positron emission tomography (PET) and magnetic resonance imaging (MRI). Many of these techniques can also be applied to human subjects in contrast to the use of radioactive or colour-labelled microspheres, which remains the gold standard of regional myocardial blood flow assessment in the experimental setting. These methodologies have been employed to investigate a variety of issues, such as the loss of endothelial-dependent relaxation after ischaemia/reperfusion and the role of oxygen radicals,[55] mechanisms of no-reflow phenomenon[13,56] and of myocardial hibernation,[57] and the impact of coronary risk factors on coronary flow reserve.[58]

4.2 Isolated heart preparations

Ex vivo isolated heart measurements of microvascular perfusion (rabbit, rat, guinea pig, and mouse) allow to accurately investigate intrinsic regulation of coronary blood flow in the absence of neuronal or hormonal influences without the confounding effects of changes in haemodynamics. Typical topics include the nitric oxide-dependent regulation of coronary blood flow[59,60] and studies of the pathways of neutrophil/vessel wall[61] and neutrophil/platelet/vessel wall[62] interactions during post-ischaemic reperfusion. Arresting isolated rat hearts with tetrodotoxin also allows for microscopic investigation of microvascular diameter responses at constant metabolic demand.[63]

4.3 Isolated vessel approaches and cultured coronary microvascular cells

Assessment of diameter/tone responses in arterioles typically isolated by surgical excision of subcutaneous tissue is a very elegant, although technically demanding, method. It can be employed to test the reactivity profile of the microcirculation with respect to endothelin, arachidonic acid metabolites, catecholamines, and other vasoactive substances. This technique has also been used in humans to examine the endothelial function/dysfunction of large and small arteries essential hypertension.[64,65] Recently, coronary vessels obtained from human atrial appendages removed during cardiopulmonary bypass were also studied.[66] One step further, cultured coronary microvascular endothelial and smooth

muscle cells allow to characterize vascular ion channels, which modulate vascular smooth muscle in microvessels, and to elucidate biochemical pathways by which endogenous vasoactive factors exert their influence.[67,68]

Regrettably, there are no animal models truly mimicking non-obstructive angina or CMD; in fact, to successfully develop such models, the underlying mechanisms need to be better known. At the moment, useful information can be gathered by investigating clinical conditions characterized by alteration of coronary artery reactivity in the absence of overt atherosclerothic stenosis.

5. Measuring myocardial microcirculatory perfusion in humans

5.1 Non-invasive screening for abnormal vascular function in humans

Commonly used tests for screening for abnormal vasomotor regulation are single photon emission computed tomography (SPECT) stress myocardial perfusion imaging for patients who can exercise and dipyridamole or adenosine-induced vasodilation in conjunction with SPECT imaging or echocardiography for patients who cannot exercise.[69–71] These techniques suffer from the limitation that only relative comparison of perfusion can be made, with no absolute estimates of blood flow. Additional methods are promising, including X-ray computed tomography (CT), echo Doppler, quantitative contrast echocardiography, and indicator-based techniques such as PET and MRI[72–75] (*Table 1*). Such measurement of tissue perfusion, however, does not assess the microcirculation independently, in that these measures interrogate the flow resistance of both the epicardial artery and the microcirculation. In the presence of fixed or dynamic (vasomotor tone) epicardial stenoses, microvascular flow resistance cannot be directly estimated. However, some insights can be drawn. Indeed, if an intervention lowers computed resistance in a stenoic vascular bed but has no effect in the same patient in a non-stenotic bed, it would be reasonable to conclude that the decline in resistance observed in the stenotic bed reflects a change in epicardial rather than microvascular resistances. Conversely, if the non-stenotic vascular bed, but not the stenosis region, exhibits a decline in resistance, it would be logical to conclude that microvascular resistance in the stenostic zone was near minimal and that epicardial resistance was unchanged by the intervention.

6. Surrogate indexes for calculating coronary microvascular resistance

There are some opportunities to develop surrogate indexes of microvascular flow resistance in the coronary arterial tree on the basis of invasively measured coronary flow parameters. These indexes are based on coronary flow parameters and hampered by the fact that myocardial flow has to be measured invasively.

6.1 Coronary and fractional flow reserve

Measuring the flow response to the downstream microvascular bed during the infusion of endothelium-dependent and endothelium-independent dilators into a coronary artery has been used to directly assess coronary microvascular function (*Figure 1*).[16,76–78] A reduced coronary flow reserve in the context of a 'normal' intravascular ultrasound or normal fractional flow reserve indicates microcirculatory dysfunction.[79] Current dual sensor pressure and flow wires allow relatively simple measurements of coronary flow reserve.[80–82] This technique may provide further insight into coronary perfusion physiology via assessment of the flow pattern (e.g. diastolic deceleration time, systolic flow reversal) and allow to calculate indexes of absolute coronary blood flow and microcirculatory resistance (taking into account systemic haemodynamics and vessel size, derived by quantitative coronary angiography or intracoronary ultrasound).

6.2 Index of microvascular resistance

Distal coronary pressure multiplied by the hyperaemic mean transit time (which is inversely correlated to absolute flow, as measured simultaneously with the coronary pressure wire) may be used to roughly estimate microvascular resistance (index of microvascular resistance).[83,84] Importantly, the pressure drop is a function of the square of flow across a coronary lesion and thus is geometrically not proportionally related. Changes in flow resistance of the vascular bed distal to the tip of the catheter can be detected. However, all resistance calculations based on this technique ignore very important physiological issues such as the true back pressure[85] in the coronary circulation and coronary capacitance and so may not always provide an accurate estimate of the parameter of interest, namely microvascular tone. In addition, preliminary data show that blood flow values cannot be readily compared among different patients

Table 1 Techniques for cardiac assessment of microvascular function

	Method	Quantification	Tracer	Spatial resolution	Recording time
Non-invasive	SPECT	None	Radio isotopes	Very low	Long
	PET	Perfusion (mL/min/g) gold standard	Radio isotopes (cyclotron-generated)	Low	Long
	CT	Perfusion (mL/min/g)	Contrast agent	Very high	Low
	MRI	Perfusion (mL/min/g)	Contrast agent	Moderate	Moderate
	Ultrasound	Perfusion (mL/min/g)	Microbubbles	High	Real time
Invasive	Doppler wire	Flow velocity (mm/s)	None	Selective assessment in target vessel territory	
	Thermo-dilution	Blood flow (mL/min)	Saline (body temperature)		
	CTFC	None	Contrast agent		

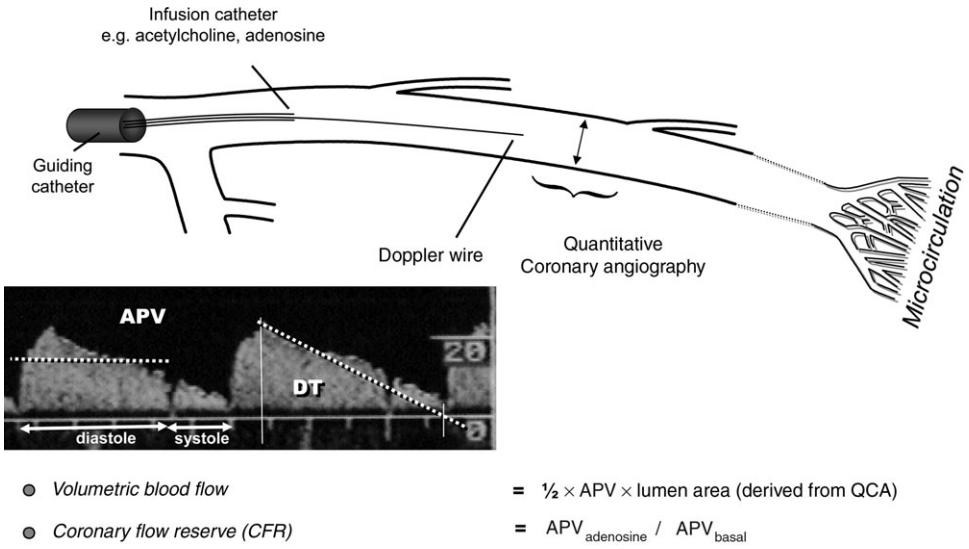

Figure 1 Set-up of intracoronary Doppler measurements of the coronary microcirculation. Blood flow velocity (APV, average peak velocity) as well as flow patterns (e.g. DT, diastolic deceleration time or systolic flow reversal) may be assessed. Volumentric blood flow may be calculated with additional assessment of coronary lumen area, approximated from quantitative coronary angiography. Vasoreactivity of the coronary microcirculation can be expressed as change of coronary blood flow in response to different stimuli (e.g. acetylcholine for endothelial stimulation or adenosine for maximal vasodilator capacity). Assuming constant epicardial vessel size, coronary flow reserve may be expressed as the ratio between maximal (adenosine-induced) and basal APV (modified, with permission from Urban & Vogel, Munich, from Schachinger V, Zeheir AM. Coronary microcirculation. Pathophysiology, clinical relevance, and importance for regenerative therapy after myocardial infarction. *Herz* 2005;**30**:641–650).

because the absolute resistance varies depending upon the tissue volume supplied by the respective artery.[83]

6.3 Thrombolysis in Myocardial Infarction Frame Count

The Corrected Thrombolysis In Myocardial Infarction (TIMI) Frame Count (CTFC) provides a simple index of coronary flow and myocardial perfusion. CTFC is an established method to provide a semiquantitative categorization of 'epicardial' blood flow, with the implicit assumption that slow flow in the absence of significant stenosis would reflect impairment of the myocardial 'microcirculatory' perfusion.[86] CTFC has been used as measure of microvascular integrity and seems to predict outcome.[87] Changes in CTFC not explained by concurrent changes in epicardial minimal lumen diameter (MLD) may be attributed to the resistance to flow at the level of coronary microcirculation. The concept of the CTFC/MLD ratio may thus be introduced as a composite measure of microvascular structure and function. Yet the value of this technique, too, needs to be fully elucidated. Measurements of blood flow using the TIMI frame count methodology require serial reproduction in patients not undergoing PCI or thrombolysis as a control group. These data are at present not available.

7. Peripheral indexes

One alternative approach to invasive cardiac procedures enabling the assessment of alterations in microvascular function and structure is based on the assumption that microvascular alterations in peripheral tissues might reflect corresponding alterations in the heart. This has been confirmed for endothelial dysfunction in patients with systemic risk factors for atherosclerosis such as diabetes, hypercholesterolaemia, or hypertension[88,89] and for patients with genetic alterations of microvascular responsiveness.[90,91] However, only few studies have systematically assessed a direct correlation between peripheral and coronary vasodilator capacity, most of them limited by comparing different entities in the peripheral and coronary vasculature with respect to vessel size and agonist tested. It was shown that the peripheral perfusion response to transient forearm ischaemia (see also what follows for details) does not correlate with dipyridamole-induced myocardial hyperaemia.[92] This might indicate different mechanisms of microvascular activation or regulation and advocate adequate care in respective extrapolations. However, peripheral techniques as summarized in what follows (*Table 2*) are less invasive and/or less expensive and time consuming compared with coronary catheterization or non-invasive screening strategies and may allow a more direct assessment of involved microvascular mechanisms.

7.1 Brachial artery post-ischaemic reflow

Brachial artery dilation in response to different stimuli can be measured with high-resolution ultrasound scanners. To test endothelium-dependent dilatation, flow changes are induced by forearm ischaemia followed by post-ischaemic hyperaemic reflow. In subjects with intact endothelial function, this typically results in a brachial artery diameter increase of $\sim$10%, and its exact quantification requires considerable technical skill and experience.[93] Data obtained are then compared with the diameter response after sublingual application of glyceryl trinitrate, an endothelium-independent dilator. This technique is non-invasive, but it assesses endothelial function in a large conduit artery and not in microvessels. Nevertheless, the results seem to correlate with responses in skin microcirculation.[94]

7.2 Venous occlusion plethysmography

In order to assess microvascular endothelial function by venous occlusion plethysmography, the brachial artery is

Table 2 Techniques for peripheral assessment of microvascular function and structure

	Method	Target vessels	Stimulus	Comments
Non-invasive	Brachial artery post-ischaemic reflow	Conduit artery	Shear stress	Validated to predict clinical outcome
	Laser Doppler flux (imaging)/ iontophoresis	Cutaneous microvessels	Vasoactive drugs	Iontophoresis: local application of a broad selection of drugs; current may have direct effect on local microvascular tone
	Clinical intravital microscopy	Cutaneous, mucosal, or retinal microvessels	None	Information on microvascular structure and function; limited range of tissues and parameters to be analysed
Invasive	Venous occlusion plethysmography (forearm blood flow)	All forearm microvessels	Vasoactive drugs	Validated to predict clinical outcome; problem: systemic recirculation of drugs
	Biopsy	Subcutaneous/ muscular microvessels	None	Analysis of microvascular structure; no functional information

cannulated for the infusion of endothelium-dependent (e.g. acetylcholine) and -independent (e.g. Na-nitroprusside) vasodilators, rendering this technique moderately invasive. It has been successfully used to demonstrate the beneficial effects of statins[95] or a third generation β-blocker[96] on endothelial function in patients with hypercholesterolaemia and hypertension, respectively. Moreover, vasodilator capacity of the peripheral microcirculation assessed by venous occlusion plethysmography predicts cardiovascular events in patients with stable coronary artery disease[97] or heart failure,[98] as well as in ACS, with those patients at highest long-term risk in whom ACS-associated impairment of the microcirculation does not recover within the following weeks.[99] Recently, finger plethysmography has been introduced, which provides further information on pulsatile flow patterns and allows the assessment of non-invasive stimuli (reactive hyperaemia).

7.3 Laser Doppler fluxmetry

Laser Doppler fluxmetry (LDF) is becoming increasingly popular for the estimation of skin microvascular blood flow.[100] The surface investigated is illuminated with a laser spot. The frequency of the laser light is altered by multiple reflections at flowing red blood cells. The resulting frequency shift in light reflected from the tissue is proportional to the product of red cell number and flow velocity (equal to a flux) in the sample region, which is usually a half sphere with a diameter of ~1 mm. Since the local perfusion in the skin is very heterogeneous, it is helpful to assess larger areas of the tissue surface by two-dimensional scanning LDF imaging. Additional information on microvascular function may be obtained by spectral analysis of the laser Doppler flux time series using wavelet transforms. Oscillations in skin perfusion with frequencies around 0.1 and 0.01 Hz are considered to reflect myogenic and endothelial activities, respectively.[101]

In combination with local drug application by iontophoresis, the LDF technique allows for non-invasive study of microvascular responses to a variety of pharmacological stimuli. Iontophoresis relies on drug molecules carrying a positive or negative charge which migrate across the skin when a respective potential is applied, in direct proportion to the current applied. Thus minute quantities of a vasoactive drug can be administered to the microvasculature under investigation in a controlled fashion, without causing systemic effects. Acetylcholine and Na-nitroprusside are typical substances to assess endothelium-dependent and -independent vasodilator responses.[102,103] In contrast to the techniques discussed earlier, this method also allows for the application of vasoconstrictors. Alpha-adrenergic vasoregulation has been analysed using iontophoretic delivery of tyramine, phentolamine, bretylium, and norepinephrine.[103] Interestingly, iontophoretically delivered urotensin II may induce vasodilation in the skin microvasculature of healthy control subjects but constriction in patients with chronic heart failure.[104]

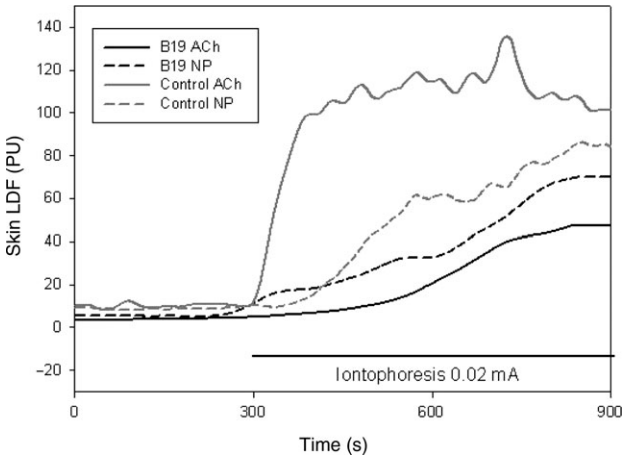

Figure 2 Laser Doppler flux tracings of skin perfusion in a patient with Parvovirus B19-induced cardiomyopathy (B19) and a healthy subject (Control) during 5 min of rest and 10 min of iontophoretic application of acetylcholine (Ach) and Na-nitroprusside (NP) at 0.02 mA.

Thus, iontophoresis in conjunction with laser Doppler flowmetry or imaging seems most attractive for detailed study of peripheral microvascular function because it is not only completely non-invasive but also allows for the assessment of endothelium-dependent and -independent vasodilator reserve and of vasoconstrictor sensitivity.

Figure 2 shows an example of laser Doppler flux tracings of skin perfusion in a patient with Parvovirus B19-induced cardiomyopathy.

7.4 Clinical intravital microscopy

In addition to functional deficits, apparent microvascular dysfunction may also be due to structural alterations of the microvasculature such as rarefaction and/or remodelling.[105,106] Evaluation of microvascular density requires direct visualization of microvascular networks by clinical intravital microscopy, which is possible only in select tissues. Capillaroscopy of the nailfold or oral tissues (gingival, sublingual, or buccal mucosa) allows to study capillary morphology, capillary density, and capillary blood velocity.[107–109] The introduction of new clinical intravital microscopes (e.g. orthogonal polarization spectral imaging) has improved image quality and ease of application and renders additional tissues such as sublingual or buccal mucosa accessible.[110] Specific image analysis approaches additionally allow the measurement of arteriolar or venular blood flow velocity[111] and pulsatility.[112]

Arteriolar morphology can also be analysed by retinography.[89] Focal or generalized luminal narrowing, as well as arteriovenous nicking, i.e. the indentation and displacement of venules by crossing arterioles owing to structural changes in the arteriolar wall, has been associated with hypertension,[113] metabolic syndrome,[114] coronary heart disease,[115] and stroke.[116] The major disadvantage of using retinography for the analysis of arteriolar remodelling is the qualitative nature of the results. A common problem of all intravital microscopy approaches lies in the difficulty of extracting quantitative data from the images, which usually requires time consuming off-line analysis of digitized video sequences.

7.5 Subcutaneous biopsy

In order to obtain quantitative data on arteriolar wall thickness to lumen ratio, histological analysis of tissue from biopsies is indispensable. In contrast to cardiac tissue, subcutaneous fat or gluteal muscle specimens can be collected in sufficient quantities with little discomfort to the patients.[117] To support the assumption that microvascular alterations occur not only in the heart or the brain but in all tissues throughout the body, results obtained in these specimens can be compared with retinography findings and with functional data obtained in the coronary and in peripheral circulations. The same specimens may also be used to investigate the mechanisms responsible for the observed morphological alterations.[118]

8. Perspectives

Although many studies have supported the role of CMD in explaining chest pain symptoms in a variety of clinical settings, convincing evidence of a causal relationship between myocardial ischaemia and CMD and of its pathophysiological mechanisms is sparse. We therefore strongly emphasize that investigations designed to elucidate the regulation of the coronary microcirculation in health and disease should incorporate the best technological advances in molecular biology, biochemistry, imaging, and physiological measurements.

Future research needs to encompass both basic and clinical investigations and to compare peripheral investigations of microcirculation with parallel measurements in the heart. Development and testing of new and improved diagnostic procedures for CMD are needed as well. Currently, the coronary microcirculation moves into the focus of new therapeutic strategies of cardiac disease driven by recent insights into, e.g. endothelial effects of drugs such as statins or ACE-inhibitors, or the restorative function of endothelial progenitor cells[119,120] and haematopoietic stem/progenitor cells. Established and experimental therapies for the prevention and treatment of microvascular dysfunction should be tested in animal and cell-based models. In addition, clinically oriented investigations will be indispensable to understand the role of the coronary microcirculation in cardiac disease and to gain more precise insights into the relationships between coronary microvascular dysfunction, inducible myocardial ischaemia, and adverse outcome. Areas such as genetic predisposition to microvascular disease in response to hypertension and/or other cardiovascular risk factors also deserve emphasis.

9. Conclusions

The authors perceive that there is an impressive lack of clinical and pathophysiological information on the role of CMD in cardiac disease. This is in part due to the gap between current experimental studies of the coronary microcirculation and clinical investigations in this area. Increased collaboration between basic science and clinical-oriented researchers should help bridging this gap and help develop improved experimental and clinical approaches to assess and treat CMD.

Conflict of interest: none declared.

Funding

None.

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