How to study coronary microvascular dysfunction and its clinical relevance

Antonio L'Abbate, Gianmario Sambuceti

CNR Institute of Clinical Physiology, Scuola Superiore di Studi Universitari S. Anna, Pisa, Italy

Key words:
Coronary microcirculation; Coronary stenosis; Myocardial ischemia.

It is now accepted that the precipitation of acute coronary syndromes and, mostly, of acute myocardial infarction often occurs in patients who do not show critical stenoses. This and other findings challenge the relevance of stenosis severity as a major pathogenetic determinant in ischemic heart disease. Thus, a large amount of research has been put forward to investigate further mechanisms, besides changes in plaque burden and stenosis severity, associated with the precipitation of ischemia. Among putative mechanisms, great attention has been paid in the last decades to the activation of the atherosclerotic plaque and its complications, while only recently the interest has been focused on the potential role of coronary microcirculation as a cofactor in many clinical manifestations of ischemic heart disease. At present, the clinical evaluation of coronary microcirculation is hampered by a number of methodological limitations while the experimental models of coronary atherosclerosis and ischemic heart disease are still far from being satisfactory. As a consequence, although an involvement of the coronary microcirculation has been suspected in a large number of clinical syndromes, its nature and mechanisms still remain largely speculative.

The present paper will try to critically review theoretical and technical approaches to the study of coronary microcirculation in ischemic heart disease with the aim at providing the reader with those elements potentially helpful to his own judgment on the matter.

(Ital Heart J 2002; 3 (4): 230-236)

Address:

Prof. Antonio L'Abbate

Istituto di Fisiologia Clinica del CNR
Area della Ricerca CNR
Via Moruzzi, 1
56124 Pisa
E-mail:
segrlabb@ifc.cnr.it

According to the classical view, the central function of coronary microcirculation is to distribute blood flow to the myocardial cells according to their need. The strict matching between myocardial oxygen consumption and coronary blood flow is warranted by changes in microvascular tone in turn finely tuned by local metabolism. This classical concept is based on a large body of evidence documenting the high oxygen extraction even at basal conditions[1], the largely aerobic nature of myocardial metabolism[2], and the powerful vasodilating property, among physiologic autacoids, of substances derived from myocardial metabolism[1]. According to this view, the precipitation of ischemia is necessarily associated with exhaustion of the compensatory vasodilation of the distal microcirculation.

However, this oversimplified model does not take into account that the maintenance of tissue homeostasis also implies a strict control of capillary pressure, as this is a major determinant of water and solute exchanges between plasma and tissue[3]. This second control can easily come into conflict with the need of increasing flow in order to match increased oxygen demand. As an example, decreased pressure downstream a severe stenosis is further lowered by any increase in flow secondary to microvascular vasodilation. A third consideration, not included into the original model, is the fact that microcirculation in the heart, as in other organs, reacts to a series of noxae or local damages such as hemorrhage, necrosis or infection addressing the response to isolate and repair the damage. These responses seem based on the interaction between vessel wall and blood cells by still largely undefined signals leading to the activation of molecular pathways which induce the adhesion of leukocytes and platelets to the vascular wall and the extrusion of blood cells into the interstitium.

Thus, modulation of the coronary vascular tone is operated by a variety of control mechanisms, aimed to tune capillary pressure, to warrant energy supply, and to protect the heart from damage. A detailed description of the pathways and interactions of these mechanisms is beyond the aims of this paper.

On the basis of the above considerations it is reasonable to suppose that the different controls on coronary microcirculation are differently distributed along the coronary tree[4]. The presence of vascular micro-

domains under the prevalent if not exclusive control of different mechanisms enhances the global regulation of flow and pressure allowing a perfect balance between oxygen need and supply without altering capillary pressure (under a variety of physiological conditions).

This concept has physiological and methodological implications, in the interpretation of both normal and abnormal coronary resistance. From a pathophysiological point of view, alteration of one of the control mechanisms can exert profound effects on the others even when their molecular pathways are preserved. For instance, although atherosclerosis does not affect adenosine-mediated vasodilation, the lack of the endothelium-mediated vasodilation of the upstream vessels can produce a drop in the microcirculatory pressure below the level necessary to maintain perfusion in some vascular units and reduce the flow response to adenosine[5]. Thus, an abnormal flow response to adenosine administration not necessarily identifies an alteration at the site of its primary action (adenosine-sensitive arterioles).

The redundancy of control mechanisms has also several implications in the methodology of studies dealing with coronary microcirculation. As the direct visualization of microvessels is precluded in patients, clinical studies of microcirculation have mostly assessed integrated parameters such as coronary blood flow, coronary pressure and coronary resistance. Alterations in one of the control mechanisms might not affect these integrated parameters. As an example, the increase in vascular tone secondary to a reduction in nitric oxide availability can be associated with normal values of coronary blood flow due to the compensatory vasodilation in most distal vascular segments[6]. Similarly, microvascular obstruction in the setting of acute coronary syndromes can be associated with even increased values of resting perfusion despite the presence of active ischemia, as documented by experimental studies, showing vasodilation of normal tissue by the spillover of adenosine from the nearby ischemic tissue[7].

Morphological study of coronary microcirculation

Histological examination of cardiac autoptic specimens has represented the basic tool for the nosographic classification of a large variety of structural vascular changes in human pathology[8,9]. However, in spite of the fact that cardiac catheterization, cardiac surgery and heart transplantation have widely expanded the opportunity for histological investigations, the information obtained in humans by direct approaches has been so far limited. Increased thickness of small arterioles has been frequently reported in various diseases (vascular hypertrophy)[10], while histological signs of microvascular atherosclerosis have been only observed in particular forms of hyperbetalipoproteinemia[11]. Moreover several authors reported the sporadic occlusion of microvessels, by platelet aggregates or microthrombi or composite material. These findings have been generally attributed to debris embolization from the atherosclerotic plaque[12]. Finally, in patients with previous myocardial infarction, microvascular alterations have been reported such as plexus or mesh of dilated small arteries, as well as large capillary-like vessels[13]. However, these findings are likely nonspecific of the atherosclerotic process but rather secondary to myocardial necrosis and fibrosis. Thus, it can be concluded that the direct investigation of coronary microcirculation by histology has not provided so far, beside vascular wall hypertrophy, sufficient elements to the documentation of autochthonous microvascular pathology. This conclusion could be different if the microvascular occlusions were interpreted as a primary rather than secondary phenomenon. This crucial point will be discussed later on.

Indirect anatomical information on coronary microarchitecture

Integrated information on the coronary microvascular anatomy can also be obtained indirectly by functional rather than morphological approaches. Under maximal vasodilation coronary blood flow is linearly correlated with aortic pressure[14]. The slope value of the correlation between flow (Y-axis) and pressure (X-axis) reflects maximal conductance that is the inverse of minimal coronary resistance. Thus the slope can be interpreted as an index of the overall architecture of coronary circulation. However, this interpretation holds on the assumption that all vessels are perfused, i.e. closure by tissue compression of otherwise anatomically normal vessels can be excluded. As the slope is function of the resistance of the integrated system of large and small vessels, in normal conditions it will mainly reflect microvascular minimal resistance, due to the small contribution of large vessel resistance to the total value. On the contrary, in the presence of coronary stenosis, minimal microvascular resistance can be assessed only if the pressure downstream the stenosis (distal coronary pressure) is used to obtain the slope.

To assess minimal coronary resistance in the clinical setting is not an easy task[14]. The value of coronary resistance is commonly obtained by the ratio between aortic (arterial) pressure and the corresponding coronary flow according to the equation

$$F = P/R$$

where F is the mean flow, P is the mean aortic pressure, and R the resistance. Using this equation, it is assumed that the driving force moving flow into the coronary circulation is the aortic pressure minus a negligible right atrial pressure.

However, several studies demonstrated that zero-flow pressure (the pressure at which the blood flow ceases) is always higher than the right atrial pressure[15]. In other words the linear correlation between flow and

pressure during maximal vasodilation intercepts at a positive value of pressure. On the basis of this observation, it has been hypothesized that the force moving coronary blood flow might actually be the pressure gradient between the aorta and myocardial tissue[16]. Thus the pressure-flow relationship becomes:

$$F = (P - Pe)/R$$

where Pe is the tissue pressure. When P decreases at a value equal to Pe in a critical segment of the vascular tree the vessel collapses and flow stops.

Taking into account the above considerations, minimal coronary resistance should be calculated by using multiple measurements of coronary blood flow at different values of arterial pressure. As a possible application of this concept, changes in arterial pressure by administration of vasoactive drugs or physical maneuvers have been proposed[17]. Alternatively the analysis of pressure-flow velocity relationship in a single diastole, using phasic blood flow monitoring by Doppler catheters has been proposed[18]. Unfortunately, both approaches are hampered by a number of limitations. The difficulty of the first approach lies in the fact that the interventions used for modifying arterial pressure may *per se* affect coronary resistance directly or indirectly through alterations of contractility, chamber size and thus wall tension. To minimize this problem, the preliminary autonomic blockade has been proposed, although it adds complexity to the study protocol. On the other hand, the single beat approach is strongly affected by vascular capacitance. In fact, as the intramyocardial blood volume is squeezed during systole into the coronary sinus and then restored during diastole, the flow recorded at the inlet of the circuit during this time reflects the refilling of the intramyocardial vascular volume and, in a variable and unknown proportion, the blood crossing the capillary network[17,19].

It is worthwhile to remind that flow, and thus resistance, values vary according to the amount of tissue perfused whose knowledge is frequently lacking. For this reason attention must be paid in the interpretation of changes in minimal resistance in the same patient in different conditions or in different patients unless a measure of specific flow (flow per unit mass of tissue) is available.

Last but not least, the problem of the certainty of reached maximal vasodilation should be considered. Standard dosages of vasodilating drugs may be insufficient in some patients thus leading to incorrect conclusion on anatomical resistance[20]. Due to this possible drawback a dose response curve should be performed when possible.

Functional study of coronary microcirculation

To evaluate microvascular function, the response of myocardial perfusion to a variety of physical or pharmacological stimuli is commonly assessed. Differently from the evaluation of minimal coronary resistance, this task can also be achieved without the measurement of myocardial blood flow. In fact, in some instances, the estimation of flow distribution throughout the myocardial walls can provide useful information, mostly when the response of this parameter to physiological or pharmacological stimulation is tested.

Single-photon myocardial scintigraphy (SPECT), positron emission tomography (PET) and more recently contrast echocardiography and magnetic resonance imaging (MRI) can noninvasively assess coronary blood flow. Characteristics and limits of each technique will be shortly discussed. As a whole, by these techniques the effect of coronary stenosis and/or microcirculatory alterations on perfusion cannot be dissected. Thus perfusion alterations can be ascribed to microcirculation only if the interference of stenosis can be excluded. For this reason the study of microcirculation by these techniques always requires the angiographic assessment of the anatomy of large coronary vessels.

SPECT allows imaging of myocardial flow distribution although the estimate of flow in absolute terms is prevented by this technique. Different is the case of PET where, in addition to distribution, repeated measurements of regional flow in ml/min/g of tissue can be obtained. At present, PET is the only established noninvasive technique able to provide such a crucial information. Similar attempts with contrast echocardiography and MRI although promising are still under evaluation. With PET the two most commonly used flow tracers are ^{13}N-ammonia[21] and ^{15}O-water[21,22]. These two tracers display an important difference: ammonia is almost entirely extracted and trapped within the myocardium; measurement of blood flow is obtained by the analysis of its uptake. By contrast, ^{15}O-water is a freely diffusible indicator; measurement of blood flow is based on the analysis of its wash-out. This difference implies that while ammonia provides the average flow in the interrogated myocardial volume, water provides the value of flow in the perfused fraction of the interrogated volume. In other words, if flow within the volume is markedly heterogeneous, water provides higher values than ammonia. Under resting conditions, the most important cause of flow heterogeneity is the mixture of fibrotic and viable tissue. However, we have recently documented, confirming experimental evidence, that the number of perfused vascular units is dependent upon perfusion pressure indicating that heterogeneity in flow does not reflect heterogeneity in tissue anatomy only[23]. Thus flow measures obtained by diffusible tracers should always be regarded as possibly affected by perfusion heterogeneity.

Contrast echocardiography is based on the intravenous injection of gaseous microbubbles that can be visualized by ultrasounds during their passage in the insonated organ. Recently, major advances have been performed with new imaging modalities such as intermittent imaging or second harmonic imaging able to

improve the signal-to-noise ratio[24]. It is important to underline that the echocontrast signal is related to the blood microvascular content rather than to flow. Thus the brightness of the contrasted myocardium reflects the number of perfused vessels independently of their flow. Accordingly contrast echocardiography has been successfully used to detect the no-reflow in patients with acute myocardial infarction, where the occurrence of microvascular obstruction plays an important role. However preliminary reports show the possibility of measuring blood flow by assessing bubble appearance time[25]. Although promising, the potential of contrast echocardiography to reach the ambitious goal of measuring both regional myocardial blood volume and flow, remains to be established. Nevertheless the advantages of high spatial and temporal resolution, lack of radiation exposure, the repeatability of contrast injection and the possibility of simultaneously detecting regional function and perfusion render this technique particularly interesting for the study of microvascular function.

In recent years there has been a growing interest in the use of MRI for the clinical assessment of myocardial perfusion[26]. The introduction of subsecond, MRI pulse sequences allows the imaging of the heart within a single beat. The acquisition of a dynamic series of such images allows the first pass of an intravenously injected bolus of contrast agent to be followed as it passes through the heart cavities and the myocardial tissue. The analysis of regional myocardial time-intensity curves provides parameters related to myocardial perfusion although the possibility to quantify flow in absolute rather than relative terms is still far from being a reality. This dynamic approach has been successfully used to evaluate myocardial perfusion in animals and in normal humans as well as in patients with coronary artery disease[26,27]. Finally, MRI also has the potentiality to measure flow velocity in the epicardial coronary arteries and hopefully to visualize large coronary arteries. The high spatial resolution and the possibility to obtain information on both myocardial perfusion and function are at present the major advantages of MRI in the study of coronary microcirculation hampered however by the lack of quantitation of myocardial flow.

By all the above imaging techniques microvascular dysfunction can be deduced on the basis of perfusion defects in absence of coronary stenosis. In this condition the administration of a vasodilating stressor is generally required to enhance the relative differences between left ventricular regions. However, a global microvascular dysfunction leading to a homogeneous reduction in vasodilating capability cannot be detected unless the absolute measurement of myocardial blood flow is possible, like is the case of PET[28].

In patients with acute coronary syndromes, mostly after revascularization, microvascular obstruction may occur. Whatever the cause of this phenomenon (embolization or autochthonous microvascular plugging)[12], the total absence of flow, although limited to minute myocardial islands, produces a signal gradient sufficient to be detected by high spatial resolution techniques such as MRI[29] and contrast echocardiography[24] without the need for vasodilator stressors.

Studying microvascular function in regions supplied by a stenotic artery

Stenosis opposites flow by adding a new resistance to the coronary tree, proportional to the amount and length of lumen reduction. When total coronary resistance is measured, according to the approaches described above, the relative contribution of stenosis and microvascular resistances cannot be ruled out unless one of the two is also measured. Several approaches have been proposed to measure stenosis resistance from its angiographic morphology, however they are not sufficiently accurate for this purpose[30]. Conversely more accurate appears the measurement of pressure gradient across stenosis and the calculation of resistance using the ratio between pressure gradient and flow. Nowadays, this approach is made possible by the use of very thin pressure wires able to cross even severe stenosis without adding substantial resistance[31]. The measurement of the input flow (by a Doppler catheter advanced just in front of the stenosis) and the simultaneous measurement of aortic and distal pressure may allow the simultaneous assessment of stenosis and microvasculature contribution to the total resistance. In this setting, the use of the Doppler catheter, as compared to the Doppler flow wire, has two major advantages: 1) it makes it possible the selective administration of vasoactive substances into the target territory, and 2) the input flow to the whole ischemic area rather than to its most distal portion can be measured.

Using this protocol design Marzilli et al.[32] assessed microvascular resistance during episodes of resting ischemia in patients with unstable angina and single vessel coronary disease. In that study, microvascular resistance during ischemia was almost 10 times higher than the one that could be obtained following adenosine administration. These data strongly support the view that ischemia is not associated with maximal vasodilation and that a "paradoxical" increase in resistance contributes to its precipitation.

With the same technique quite similar results were also obtained in patients with chronic angina pectoris and single vessel disease[33]. In these patients ischemia was induced by atrial pacing tachycardia and was consistently associated with a reduction in blood flow and no significant changes in distal coronary pressure. When adenosine was administered through the Doppler catheter during pacing sustained ischemia, a marked flow increase was observed associated in all patients with a fall in distal coronary pressure and in several cases with a reduction in ST-segment depression at

ECG. Thus, selective vasodilation of the ischemic vascular bed was followed by a reduction in microvascular resistance and an amelioration of electrical signs of ischemia. Alpha-receptor blockade by phentolamine did not affect this paradoxical vasoconstriction which, instead, was fully prevented by angioplasty.

Altogether these data seem to indicate that a microcirculatory increase in resistance plays a role in the precipitation of ischemia and that this paradoxical response is, in some way, triggered by the interaction between the hydraulic effects of epicardial stenosis and microvasculature. Actually, several experimental data already reported an increase in microvascular resistance in models of prolonged ischemia by coronary constriction[34] or marked hypotension secondary to hemorrhagic shock[35]. All these data contrast the classical concept that metabolic regulation of myocardial perfusion induces a maximal vasodilation under conditions of restricted flow. Nevertheless, it should be considered that the drop in pressure at the inlet of microcirculation, due to stenosis, produces a series of readjustments in microvascular resistance. A large literature suggests the presence of an intrinsic control of vasomotor tone aimed to maintain constant the pressure in the capillary network as already discussed. A metabolic vasodilation, in regions supplied by a severely stenotic coronary artery, would imply a further fall in pressure that might alter this equilibrium. Vasoconstriction in the downstream venules might maintain capillary pressure through a homogeneous reduction in flow. However, ischemia is far from being a homogeneous phenomenon as suggested by a variety of studies documenting the increase in the heterogeneity of both flow and metabolism[35,36]. Thus, resistance might increase in only a portion of the arterial tree with the exclusion of some vascular units while others might be perfused at normal pressure and still maintain a flow reserve unmasked by adenosine administration.

This concept has important physiological and methodological implications. As previously discussed on the use of ^{13}N-ammonia or ^{15}O-water as flow tracers with PET, in the presence of high heterogeneity, diffusible indicators such as water or inert gases tend to overestimate blood flow as their wash-out preferentially trace the better perfused vascular units. This problem is irrelevant when tracers trapped within the myocardium such as microspheres, technetium labeled agents or ammonia are used to assess the mean flow of the interrogated myocardium. Although this difference seems somewhat academic, it falls into clinical practice when the pathophysiology of chronic dysfunction is considered. In this setting, the mixture of fibrotic with normal viable tissue highly contributes to heterogeneity. In agreement with theory, measurements of flow with diffusible tracers provide higher (normal) values with respect to methods using ammonia. Thus according to some methods dysfunctioning myocardium is normally perfused while according to others it is hypoperfused[37,38].

Along this line the possible existence of flow heterogeneity in apparently homogeneous conditions like downstream a severe stenosis in the absence of previous myocardial infarction should also be considered. To assess flow heterogeneity is a difficult task due to the limited spatial resolution of the current imaging techniques. Starting from the consideration that this phenomenon cannot be realistically addressed by any clinical imaging technique, whether confined at microscopic level, we tried to overcome the problem by a nonimaging although invasive approach. This was accomplished by the simultaneous measurement of both total and specific coronary blood flow (flow per unit mass of perfused myocardium) in a left ventricular region perfused by a severely stenotic coronary artery in patients with stable effort angina and single coronary vessel disease undergoing percutaneous coronary angioplasty[23]. A Doppler catheter, positioned immediately upstream the stenosis, was used to measure total flow while specific flow was obtained by the wash-out rate of radioactive Xenon after its intracoronary injection. Perfused myocardial volume before and after percutaneous coronary angioplasty was thus calculated as the ratio between total and specific blood flow. Results from this study documented that following coronary angioplasty the perfused tissue fraction increased up to 6 times and was linearly correlated with the increase in driving coronary pressure. Thus, in patients with coronary stenosis a redistribution of microvascular resistance and flow occurs that is dependent upon the pressure drop in the coronary microcirculation.

Increased microvascular resistance in acute myocardial infarction: the elusive role of vasoconstriction

In the previous sections we focused on the clinical evidence of increased microvascular resistance during brief periods of ischemia and on the role of distal coronary pressure on vascular recruitment even in the absence of ischemia. Studies on (total) coronary resistance in acute myocardial infarction have been generally limited to the reperfusion phase and its follow-up. The frequent observation of persistent increased total resistance following pharmacological or mechanical reopening of the occluded coronary artery has been generally attributed to suboptimal vessel recanalization, to ischemia-reperfusion damage[23] or to embolic material[14] and vasoconstrictive substances[39,40] originated at the level of the atherosclerotic plaque. This radicated view seems supported by the histological report of microvascular plugs containing platelets, red and white blood cells and lipids. On this basis, it has been proposed that limiting platelet aggregation and thrombus formation might prevent microvascular embolization thus reducing the extent of myocardial necrosis[41]. This hypothesis has been confirmed by several studies

on the use of the antagonists of glycoprotein IIb/IIIa in coronary percutaneous interventions[42,43]. Although these clinical results seem to support the view that prevention of thrombus formation at the plaque level can decrease microembolization, more recent experimental evidence could question this interpretation and alternatively suggests an autochthonous origin of microvascular occlusion. Increased expression of adhesion molecules and in particular of P-selectin at the microvascular level has been documented in animal models of ischemia[44], particularly in the presence of reduced endothelial nitric oxide synthase activity[45]. Accordingly, the use of P-selectin antagonists prevented microvascular plugging and reduced infarct size. These and other experimental findings lead to the suggestive hypothesis of a microcirculatory inflammatory response to ischemia and reperfusion responsible for both vasoconstriction and mechanical obstruction of microvessels.

Preliminary results from our laboratory, in patients with unstable angina, seem to support this hypothesis[46]. Using the Doppler catheter and pressure wire technique to separately assess stenosis and microvascular resistance it was shown that the administration of abciximab had little effect on the stenotic arterial segment while significantly reduced basal and postadenosine microvascular resistance. Moreover, abciximab abolished microvascular vasoconstriction and myocardial ischemia induced by hyperventilation in the same patients. In another study in patients with acute infarction undergoing primary angioplasty, Marzilli et al.[47] pretreated with local administration of adenosine the ischemic myocardium immediately before recanalization. As compared to the non-treated group, adenosine markedly reduced the occurrence of no reflow and improved functional recovery and outcome. The results of this study strongly suggest that administration of drugs that "target" coronary microcirculation can improve the results of therapy in the setting of acute ischemic syndromes. These findings however do not allow us to draw any conclusion on the mechanism of action of adenosine. On one side, it might prevent the microvascular constriction observed in all clinical models of myocardial ischemia through its vasodilatory effect on small vessels. On the other side, it might inhibit P-selectin expression in the ischemic myocardium with beneficial effects on blood flow, contractile dysfunction and lactate uptake as recently documented in animal experiments[44]. This latter mechanism might represent an alternative or complementary interpretation of the role of adenosine in acute coronary syndromes.

Altogether these data confirm that coronary microcirculation actively participates in the precipitation of myocardial ischemia. However, the mechanisms underlying microvascular alterations are still largely undefined. On the one hand a primary reaction to reduced intravascular pressure, ischemia and myocardial damage might occur; on the other a preexisting dysfunction of the coronary microvascular tone, as described in regions remote from stenosis or infarction perfused by angiographically normal coronary arteries[28,48,49] might also play a role in the onset and evolution of ischemia.

Conclusions

Despite technical and conceptual difficulties in the study of microcirculation, it seems well established that microcirculation contributes to the pathogenesis and outcome of acute and chronic coronary syndromes due to either an inappropriate vasoconstriction or an inflammatory response to a variety of noxae, including, but not limited to, ischemia and reperfusion. It is worth to further explore these pathogenetic mechanisms as microvascular dysfunction may become a new important pharmacological target.

References

1. Gregg D, Fisher LC. Blood supply to the heart. In: Handbook of physiology. Section II. Washington, DC: American Physiological Society, 1963.
2. Monroe RG. Myocardial oxygen consumption during ventricular contraction and relaxation. Circ Res 1964; 14: 293-9.
3. Landis EM, Pappenheimer JR. Exchange of substances through capillary walls. In: Hamilton WF, Dow P, eds. Handbook of physiology. Vol II, Section 2. Bethesda, MD: American Physiological Society, 1927: 961-1034.
4. Kuo L, Davis MJ, Chilian WM. Longitudinal gradients for endothelium-dependent and independent vascular responses in coronary microcirculation. Circulation 1995; 92: 518-25.
5. Chilian WM, Eastham CL, Layne SM, Marcus ML. Small vessel phenomena in the coronary microcirculation: phasic intramyocardial perfusion and coronary microvascular dynamics. Prog Cardiovasc Dis 1988; 31: 17-38.
6. Smith TP Jr, Canty JM Jr. Modulation of coronary autoregulatory responses by nitric oxide. Evidence for flow-dependent resistance adjustments in conscious dogs. Circ Res 1993; 73: 232-40.
7. Dorge H, Neumann T, Behrends M, et al. Perfusion-contraction mismatch with coronary microvascular obstruction: role of inflammation. Am J Physiol 2000; 279: H2587-H2592.
8. James TN. The spectrum of diseases of small coronary arteries and their physiologic consequences. J Am Coll Cardiol 1990; 15: 763-74.
9. Rose AG. Diseases of the small coronary arteries. In: Silver MD, ed. Cardiovascular pathology. 2nd edition. New York, NY: Churchill Livingstone, 1991: 565-93.
10. Schwartzkopff B, Motz W, Frenzel H, Vogt M, Knauer S, Strauer BE. Structural and functional alterations of the intramyocardial coronary arterioles in patients with arterial hypertension. Circulation 1993; 88: 993-1003.
11. Vlodaver Z, Neufeld HN, Edwards JE. Pathology of angina pectoris. Circulation 1972; 46: 1048-64.
12. Topol EJ, Yadav JS. Recognition of the importance of embolization in atherosclerotic vascular disease. Circulation 2000; 101: 570-80.
13. Farrer-Brown G. Normal and diseased vascular pattern of myocardium of human heart. II. Pattern seen with fibrosis of the left ventricle. Br Heart J 1968; 30: 537-45.

14. Hoffman JIE. Maximal coronary flow and the concept of coronary reserve. Circulation 1984; 70: 153-9.
15. Bellamy RF. Diastolic coronary artery pressure-flow relations in the dog. Circ Res 1978; 43: 92-101.
16. Downey JM, Kirk ES. Inhibition of coronary blood flow by a vascular waterfall mechanism. Circ Res 1975; 36: 753-60.
17. Eng C, Jentzer JH, Kirk ES. The effects of coronary capacitance on the interpretation of diastolic pressure-flow relationships. Circ Res 1982; 50: 334-41.
18. Mancini JGB, McGillem MJ, DeBoe SF, Gallagher KP. The diastolic hyperemic flow versus pressure relation. Circulation 1989; 80: 941-50.
19. Spaan JAE, Breuls NPW, Laird JD. Diastolic-systolic coronary flow differences are caused by intramyocardial pump action in the anesthetized dog. Circ Res 1981; 49: 584-93.
20. L'Abbate A, Camici P, Trivella MG, et al. Time-dependent response of coronary flow to prolonged adenosine infusion: doubling of peak hyperaemic flow. Cardiovasc Res 1981; 15: 282-6.
21. Czernin J, Porenta G, Brunken R, et al. Regional blood flow, oxidative metabolism and glucose utilization in patients with recent myocardial infarction. Circulation 1993; 88: 884-95.
22. Bergmann SR, Fow KAA, Rand AL, et al. Quantification of regional myocardial blood flow in vivo with $H_2{}^{13}O$. Circulation 1984; 70: 724-33.
23. Sambuceti G, Marzilli M, Mari A, et al. Clinical evidence for myocardial derecruitment downstream from severe stenosis: pressure-flow control interaction. Am J Physiol 2000; 279: H2641-H2648.
24. Bolognese L, Antoniucci D, Rovai D, et al. Myocardial contrast echocardiography versus dobutamine echocardiography for predicting functional recovery after acute myocardial infarction treated with primary coronary angioplasty. J Am Coll Cardiol 1996; 28: 1677-83.
25. Wei K, Ragosta M, Thorpe J, Coggins M, Moos S, Kaul S. Noninvasive quantification of coronary blood flow reserve in humans using myocardial contrast echocardiography. Circulation 2001; 103: 2560-5.
26. Lombardi M, Jones RA, Wesby J, et al. MRI for the evaluation of regional myocardial perfusion in an experimental animal model. J Magn Reson Imaging 1997; 7: 987-95.
27. Lauerma K, Virtanen KS, Sipila LM, Hekali P, Aronen HJ. Multislice MRI assessment of myocardial perfusion in patients with single-vessel proximal left anterior descending coronary artery disease before and after revascularization. Circulation 1997; 96: 2859-67.
28. Sambuceti G, Marzullo P, Giorgetti A, et al. Global alteration in perfusion response to increasing oxygen consumption in patients with single vessel coronary artery disease. Circulation 1994; 90: 1696-705.
29. Wu KC, Zerhouni EA, Judd RM, et al. Prognostic significance of microvascular obstruction by magnetic resonance imaging in patients with acute myocardial infarction. Circulation 1998; 97: 765-72.
30. Demer L, Gould KL, Kirkeeide R. Assessing stenosis severity: coronary flow reserve, collateral function, quantitative coronary arteriography, positron imaging, and digital subtraction angiography. A review and analysis. Prog Cardiovasc Dis 1988; 30: 307-22.
31. Pijls NH, De Bruyne B, Peels K, et al. Measurement of fractional flow reserve to assess the functional severity of coronary-artery stenoses. N Engl J Med 1996; 334: 1703-8.
32. Marzilli M, Sambuceti G, Fedele S, L'Abbate A. Coronary microcirculatory vasoconstriction during ischemia in patients with unstable angina. J Am Coll Cardiol 2000; 35: 327-34.
33. Sambuceti G, Marzilli M, Fedele S, Marini C, L'Abbate A. Paradoxical increase in microvascular resistance during tachycardia downstream from a severe stenosis in patients with coronary artery disease: reversal by angioplasty. Circulation. 2001; 103: 2352-60.
34. Gorman MV, Sparks HV. Progressive coronary vasoconstriction during relative ischemia in canine myocardium. Circ Res 1982; 51: 411-20.
35. Miyazaki K, Hori S, Inoue S, et al. Characterization of energy metabolism and blood flow distribution in myocardial ischemia in hemorrhagic shock. Am J Physiol 1997; 273: H600-H607.
36. Austin RE, Aldea GS, Coggins DL, Flynn AE, Hoffman JIE. Profound spatial heterogeneity of coronary reserve. Circ Res 1990; 67: 319-31.
37. Marinho NV, Keogh BE, Costa DC, Lammerstma AA, Ell PJ, Camici PG. Pathophysiology of chronic left ventricular dysfunction. New insights from the measurement of absolute myocardial blood flow and glucose utilization. Circulation 1996; 93: 737-44.
38. Gewirtz H, Fischman AJ, Abraham S, Gilson M, Strauss HW, Alpert NM. Positron emission tomographic measurements of absolute regional myocardial blood flow permits identification of nonviable myocardium in patients with chronic myocardial infarction. J Am Coll Cardiol 1994; 23: 851-9.
39. Ambrosio G, Weisman HF, Mannisi JA, Becker LC. Progressive impairment of regional myocardial perfusion after initial restoration of postischemic blood flow. Circulation 1989; 80: 1846-61.
40. Wilson RF, Lesser JF, Laxson DD, White CW. Intense microvascular constriction after angioplasty of acute thrombotic arterial lesions. Lancet 1989; 1: 807-11.
41. Topol EJ, Byzova TV, Plow EF. Platelets, GPIIb-IIIa blockers. Lancet 1999; 353: 227-31.
42. Randomised placebo-controlled and balloon-angioplasty-controlled trial to assess safety of coronary stenting with use of platelet glycoprotein-IIb/IIIa blockade. The EPISTENT Investigators. Evaluation of Platelet IIb/IIIa Inhibitor for Stenting. Lancet 1998; 352: 87-92.
43. Heeschen C, Hamm CW, Goldmann B, Deu A, Langenbrink L, White HD. Troponin concentrations for stratification of patients with acute coronary syndromes in relation to therapeutic efficacy of tirofiban. PRISM Study Investigators. Platelet Receptor Inhibition in Ischemic Syndrome Management. Lancet 1999; 354: 1757-62.
44. Minamino T, Kitakaze M, Asanuma H, et al. Endogenous adenosine inhibits P-selectin-dependent formation of coronary thromboemboli during hypoperfusion. J Clin Invest 1998; 101: 1643-53.
45. Lefer D, Jones S, Girod S, et al. Leukocyte-endothelial cell interactions in nitric oxide synthase-deficient mice. Am J Physiol 1999; 276: H1943-H1950.
46. Sambuceti G, Marzilli M, Testa R, Fedele S. Platelet GP IIb/IIIa receptor blockade and coronary resistance in unstable angina. (abstr) Eur Heart J 2000; 21 (Suppl): 3549.
47. Marzilli M, Orsini E, Marraccini P, Testa R. Beneficial effects of intracoronary adenosine as an adjunct to primary angioplasty in acute myocardial infarction. Circulation 2000; 101: 2154-9.
48. Uren NG, Crake T, Lefroy DC, de Silva R, Davies GJ, Maseri A. Reduced coronary vasodilator function in infarcted and normal myocardium after myocardial infarction. N Engl J Med 1994; 331: 222-7.
49. Gibson CM, Ryan KA, Murphy SA, et al. Impaired coronary blood flow in nonculprit arteries in the setting of acute myocardial infarction. The TIMI Study Group. Thrombolysis in Myocardial Infarction. J Am Coll Cardiol 1999; 34: 974-82.