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REVIEW

# Myocardial perfusion echocardiography and coronary microvascular dysfunction

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## Abstract

Our understanding of coronary syndromes has evolved in the last two decades out of the obstructive atherosclerosis of epicardial coronary arteries paradigm to include anatomo-functional abnormalities of coronary microcirculation. No current diagnostic technique allows direct visualization of coronary microcirculation, but functional assessments of this circulation are possible. This represents a challenge in cardiology. Myocardial contrast echocardiography (MCE) was a breakthrough in echocardiography several years ago that claimed the capability to detect myocardial perfusion abnormalities and quantify coronary blood flow. Research demonstrated that the integration of quantitative MCE and fractional flow reserve improved the definition of ischemic burden and the relative contribution of collaterals in non-critical coronary stenosis. MCE identified no-reflow and low-flow within and around myocardial infarction, respectively, and predicted the potential functional recovery of stunned myocardium using appropriate interventions. MCE exhibited diagnostic performances that were comparable to positron emission tomography in microvascular reserve and microvascular dysfunction in angina patients. Overall, MCE improved echocardiographic evaluations of ischemic heart disease in daily clinical practice, but the approval of regulatory authorities is lacking.

**Key words:** Contrast echocardiography; Myocardial perfusion; Myocardial ischemia; Microvascular angina; Coronary flow



**Core tip:** Diagnostic work-up of coronary heart disease is evolving to include evaluations of the coronary microcirculation in addition to the imaging of obstructive atherosclerosis of coronary arteries and its eventual effects. Functional assessments of coronary microvasculature have become the challenge. Myocardial contrast echocardiography (MCE) emerged as a promising tool several years ago to detect myocardial perfusion abnormalities and quantify coronary blood flow. MCE compared favorably with other expensive techniques, and it accurately evaluated coronary microvascular

reserve and dysfunction in research studies. However, its daily use in clinical practice is not established. Therefore, the future of this technique is questionable.

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## INTRODUCTION

The past paradigm of ischemic heart disease was the relationship between myocardial ischemia and obstructive atherosclerosis of the epicardial coronary arteries[1]. Coronary angiography was the gold standard to evaluate the severity and extent of coronary artery disease (CAD). Clinical data in the last two decades challenged this paradigm and shifted the attention to the possible role of anatomical and functional abnormalities of the coronary microcirculation throughout the clinical spectrum of myocardial ischemia.

Functional assessments of coronary microvasculature have become the challenge. Direct visualization of the coronary microcirculation is not possible with any currently available technique, but descriptions of its function are possible. The thrombolysis in myocardial infarction (TIMI) frame count is a qualitative method to evaluate coronary blood flow[2], and intracoronary thermodilution and intracoronary Doppler wire, which are based on thermal dilution curves and the Doppler principle, respectively, measure myocardial blood flow (MBF). Transthoracic Doppler echocardiography is a noninvasive technique that is widely used to measure coronary blood flow reserve primarily in the left anterior descending coronary (LAD) artery territory[3].

The well-documented diagnostic accuracy of single-photon emission computer tomography (SPECT) myocardial perfusion imaging for CAD[4] promoted its widespread clinical use[5]. Myocardial perfusion is generally evaluated in a qualitative or semi-quantitative manner[6] that suffers from several limitations: Attenuation and Compton scatter effects; the plateau effect of $^{99m}$Tc uptake, which limits the detection of further increase in flow, and the limited spatial resolution[7]. Recent technical and methodological advances, such as dynamic SPECT using a SPECT/computed tomography camera[8-10], allow measurements of absolute MBF and its reserve, but the approximately 12-mSv exposure to the patient limits its clinical use[11].

Positron-emission tomography (PET) allows the calculation of blood flow per unit of mass, which quantifies microvascular function[12]. Contrast-enhanced cardiac magnetic resonance (CMR) imaging is the other technique that accurately quantifies MBF[13,14].

Myocardial contrast echocardiography (MCE) is a bedside and relatively low-cost tool to detect myocardial perfusion abnormalities and quantify regional and global coronary blood flow. The clinical use or MCE is limited despite growing evidence to support its reliability[15].

This review discusses research results and the established settings in which MCE use may support clinical decision making.

## MCE PROTOCOLS

### *Ultrasound contrast agents*

The following contrast agents are commercially available: Optison (Amersham Health AS, Oslo, Norway), Definity (Bristol-Myers Squibb Billerica, Massachusetts), and Sonovue (Bracco, Milan, Italy). These agents consist of a shell of albumin, lipids or galactose filled with a gas to form microspheres smaller than 10 μm. The shell allows the low diffusible and low solubility gas the resistance to intravascular pressure and the ability to share erythrocyte rheology in the intravascular compartment, including the transpulmonary passage. Therefore, microspheres reach the left heart cavities and opacify the left ventricle and myocardium.

### *Physical principles*

Microspheres are strong ultrasound scatterers. Microsphere behavior in an ultrasound field depends on the energy of the ultrasound source. Very low-energy ultrasound induces linear oscillations of the microspheres, and its fundamental frequency is reflected. Non-linear oscillations of microspheres emerge with increasing incident ultrasound energy when compression and rarefaction waves of variable magnitude are produced and generate harmonics, *i.e.*, ultrasounds of higher-order frequencies than the fundamental frequency. High-intensity ultrasound is used in medical imaging and disrupts microbubbles (Figure 1). In contrast, enhanced ultrasound imaging uses low-energy ultrasound in which the myocardium primarily exhibits linear responses and generates few harmonic frequencies, as opposed to the contrast agent. Selective reception and amplification of harmonic echoes allows sensitive detection of the contrast with good signal-to-noise ratio.

### *Imaging modalities*

High-intensity ultrasounds (mechanical index-MeI > 0.3) in standard echocardiographic imaging destroy microbubbles. The acquisition of one ECG-triggered systolic frame every several cardiac cycles improves the contrast effect by reducing microbubble destruction, and it allows time for the replenishment of myocardial microvasculature with contrast for each subsequent triggered frame. Low-MeI real-time contrast echocardiography greatly improved left ventricular and myocardial opacification compared to low-frame rate high-MeI intermittent contrast imaging. Contrast enhancement in pulse inversion Doppler technique is obtained by the transmission of two pulses of the same amplitude and inverted phase: The subtraction of reflected ultrasounds of a linear scatterer (the myocardium) generates

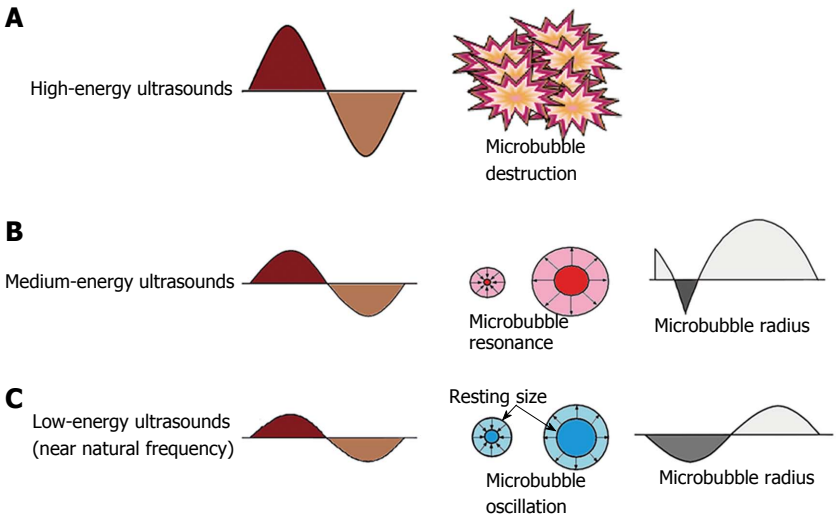


**Figure 1 Behavior of microbubbles in an ultrasonic field.** An acoustic wave generated by an ultrasound system consists of alternating high and low pressures: The positive pressure compresses the microbubble, and the negative pressure expands it. High-energy ultrasound (within the energy levels used for diagnostic echocardiographic imaging) destroys microbubbles (A); Intermediate energy ultrasound triggers asymmetrical nonlinear oscillations of microbubbles so that the magnitude of compression and rarefaction waves are not the same with each oscillation, and frequencies other than (*e.g.*, multiple of) the intrinsic fundamental frequency are generated (B); Low-energy ultrasound causes microbubbles to oscillate linearly, which reflects ultrasound at their intrinsic fundamental frequency (C).

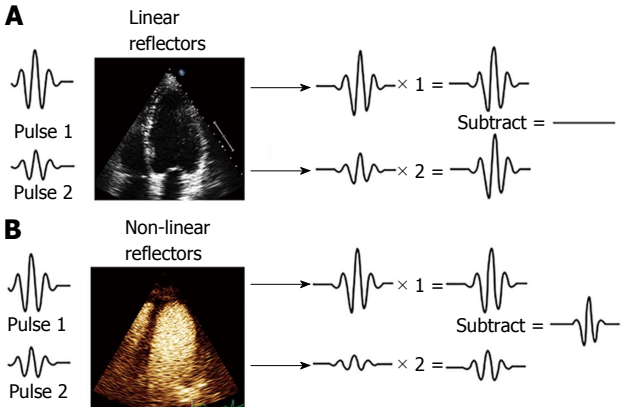


**Figure 2 Pulse inversion harmonic imaging signal processing: Effects on linear and non-linear reflectors.** This technique consists of the transmission of a first pulse and a second inverted replica of the first pulse. Any linear target, such as blood in conventional echocardiography, responds equally to positive and negative pressures (A) and reflects back to the transducer equal but opposite echoes, which will be canceled (blood displayed in black in 2-dimensional echocardiography); B: Pulse 1 and pulse 2 excite microbubbles generating fundamental and higher order harmonic responses with different phases that constructively add.

no signal, and the summated signals result from nonlinear scatterers (microspheres) (Figure 2). Other multi-pulse techniques were developed to improve the signal-noise ratio. The amplitude of one pulse can be increased (Power modulation, Philips, Andover, Massachusetts) (Figure 3), or amplitude and phase can be modulated (Cadence™ contrast pulse sequencing, Siemens Acuson Sequoia; Mountain View, California) to generate ultraharmonic oscillations. Both techniques exhibit excellent spatial resolution to assess myocardial contrast in real time.

### *Qualitative assessment*

Low flow constant contrast infusion produces homogeneous opacification of the myocardial wall in patients without significant stenosis of epicardial coronary arteries because new microbubbles uniformly replaced those that are destroyed by a flash of high-intensity ultrasound. In contrast, the rate of contrast replenishment is reduced at rest in myocardial regions where microcirculation is damaged by previous infarction or during stress in regions supplied by a significantly stenosed epicardial vessel. Myocardial flow increase during pharmacological or physical stress enhances regional differences of myocardial opacification and allows the quantification of regional perfusion as normal, reduced, or severely reduced.

### *Quantitative assessment*

The basis for myocardial flow quantification in MCE studies is the complete intravascular compartmentalization of microbubbles, which allows reaching a steady state concentration of microbubbles during continuous infusion. Replenishment of myocardial microvasculature after complete destruction of microbubbles using a flash of high-intensity ultrasounds may be assessed as a time-intensity curve. Intensity values "y" fitted to a monoexponential function: $y = A \times (1-e^{-\beta t})$ gives a measure of mean myocardial microbubble velocity ($\beta$), whereas the microvascular cross-sectional area is obtained from the plateau value of the replenishment curve (A). The product of A and $\beta$ represents MBF.

Vogel *et al*[16] proposed the use of ratio of myocardial video intensity to the adjacent left ventricular cavity as an adjustment for the inhomogeneous contrast enhancement of the myocardium due to attenuation or other technical factors. An excellent correlation was found between absolute MBF (mL/min per gram of myocardium) measured with MCE and the values obtained using PET.

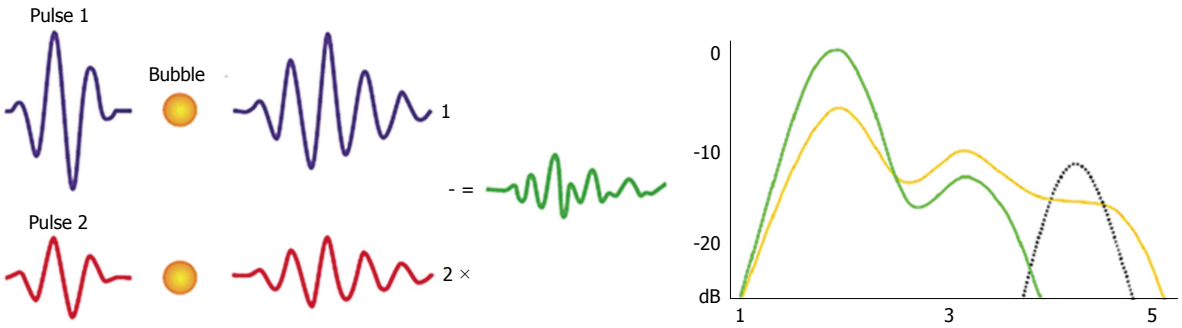


**Figure 3 Power modulation imaging.** This technique changes the amplitude of each successive pulse in a group of transmitted pulses and detects the differential nonlinear responses generated from two different excitations. Microbubbles' response to multipulse cancellation technique produces ultra-harmonic oscillations that are detected in the field of higher frequencies.

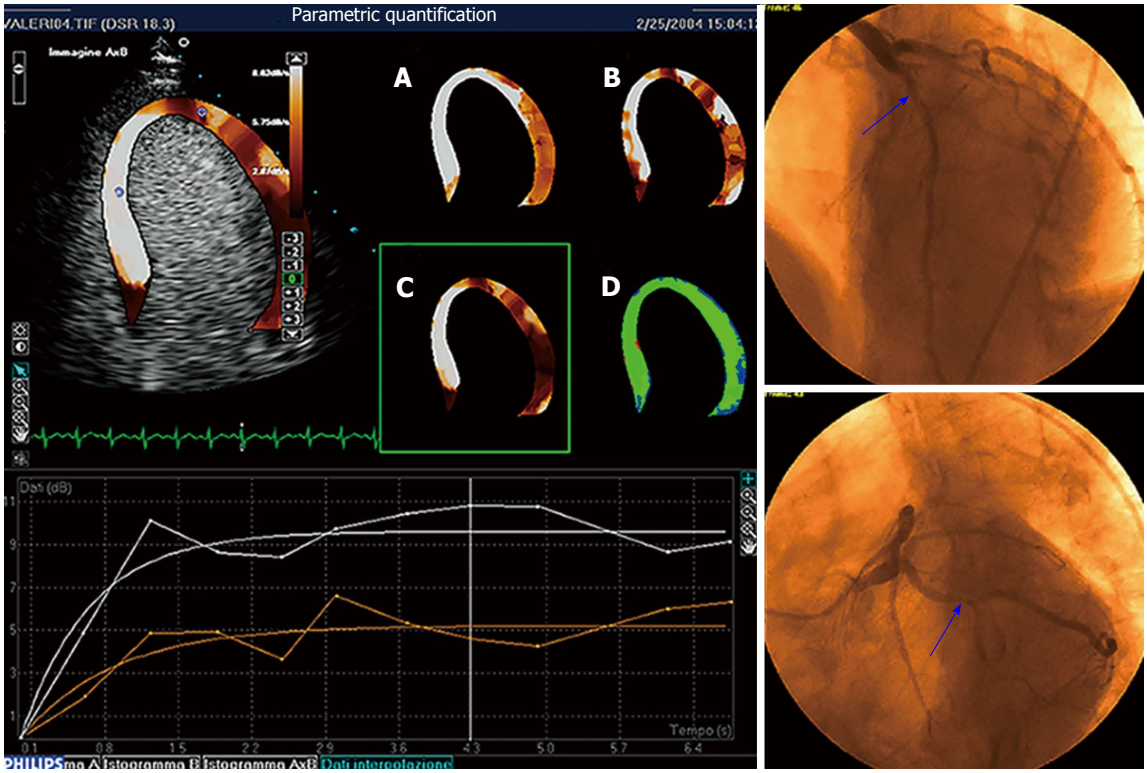


**Figure 4 Parametric quantification of myocardial perfusion.** Critical stenosis of the circumflex artery is present at coronary angiography (right panels). Parametric images of peak dipyridamole stress echo reported in panels A through D represent, respectively, the plateau value A of the contrast replenishment curve, the slope β of the replenishment curve, the A × β value, and the goodness of fitting. The A × β value parametric imaging is also displayed superimposed onto the apical four-chamber view in the top left panel. Replenishment time-course curves (sampling and interpolation) relative to the septum (white curves) and the apico-lateral wall (red curves) are reported in the graph at the bottom. Flow is reduced in the territory perfused by circumflex artery.

A fast and easy method to quantify MCE is represented by parametric images (Figure 4), which furnished visual information on the maximal intensity of contrast (A), rate of replenishment (β) and quality of acquisition[17]. Four, two and three chamber apical acquisitions of MCE create feasible computations of MBF.

## CORONARY ANATOMY AND REGULATION

A detailed description of coronary anatomy and regulation is beyond the scope of this review. However, some features must be described to appreciate the potential of MCE. Figure 5 shows the complexity of coronary anatomy. Coronary circulation comprises the large epicardial conduit vessels and resistance vessels. Resistance to flow is very low in conduit vessels and progressively increases as resistance vessel diameter decreases to the arteriolar bed (from 300 to 100 μm). The so-called "coronary driving pressure", *i.e.*, the pressure gradient between the aortic root and the right atrium, is the main determinant of blood flow across the myocardium. The coronary driving pressure under normal conditions reduces little, if any, along the epicardial conduit vessels, but it declines progressively along the microvasculature, particularly in 300-100 μm

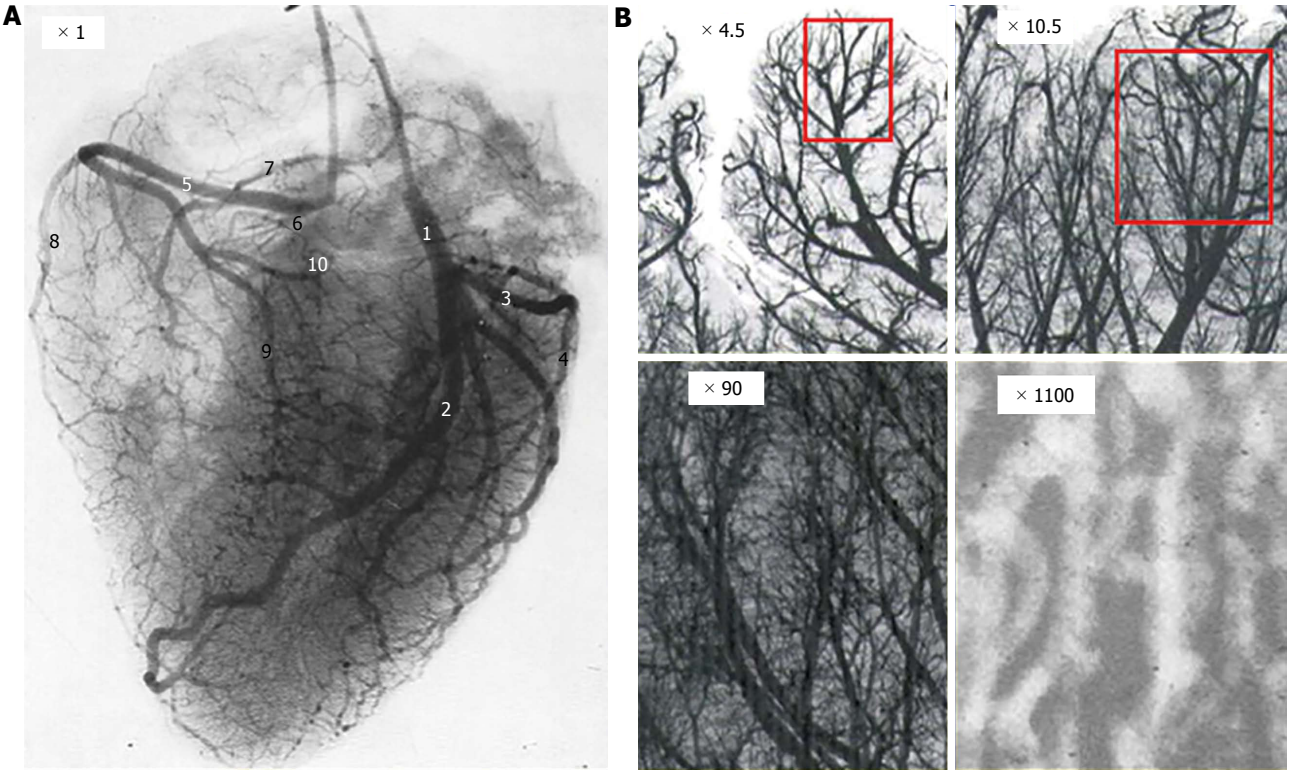


**Figure 5 Coronary artery tree arborization.** A: Angiographic still frame of left and right coronary arteries of an isolated human heart after injection of radiopaque dye; B: Progressive magnification of a region of interest showing the coronary tree fractal anatomy.

diameter arterioles, until reaching the 20-30 mmHg level. Extravascular resistive forces, which are related to the development of left ventricular systolic pressure, inotropism of the myocardium, and heart rate are additional determinants of the resistance to flow across the myocardium.

Coronary circulation has two main characteristics. First, oxygen extraction at rest is approximately 75%, but coronary venous oxygen saturation during strenuous exercise may decrease to approximately 10%. The increase in flow needed to meet the 4- to 5-fold increase in oxygen demand is accomplished by vasodilation of resistance coronary arteries, which is mediated by mechanisms that are intrinsic to the vascular wall and metabolic and neuro-humoral factors[18]. Second, coronary flow is pulsatile, *i.e.*, it is high in diastole and low in systole.

Coronary arterial pressure and myocardial oxygen consumption are the major determinants of coronary flow, but coronary autoregulation allows the flow to be relatively independent of the driving pressure at fixed oxygen consumption level, whereas at any given coronary arterial pressure a metabolic adaptation of coronary flow to oxygen requests may occur[19].

The hemodynamic impact of a focal diameter reduction of epicardial vessels is modulated by the vasodilation of resistance coronary arteries. Maximal flow reduces at maximal vasodilation because coronary perfusion pressure decreases with the square of flow through the stenosis. This law of coronary hemodynamics is the basis for dynamic assessment of coronary stenosis by pharmacological or physical stress that can elicit a reversible perfusion defect and eventually a transient regional asynergy of contraction.

Experimental canine models, in which external constriction was applied to normal coronary arteries, led to the concept of "critical" coronary stenosis. In these studies, an 85% diameter narrowing caused a fall in resting coronary flow, but a 50% diameter narrowing reduced maximum coronary flow. In surgical decision-making, the angiographic criterion of > 70% coronary stenosis was appropriate to define eligibility for revascularization.

However, this paradigm failed to describe the correlation between coronary flow reserve (CFR) and percent coronary stenosis in human studies[20], and it does not consistently identify patient groups whose prognosis can be improved by primary coronary interventions. A further confirmation of the dissociation between anatomic and functional severity of stenosis came from the poor correlation between quantitative percent stenosis on invasive or computed tomography coronary angiograms and fractional flow reserve (FFR) using pressure flow wire[21]. FFR is a validated, reproducible measurement of relative CFR. The reference model for FFR is the hyperemic pressure-flow relation with the assumptions that a discrete stenosis induces proportional changes of perfusion pressure and flow, and downstream of a stenosis minimal coronary resistance equals that of a normally perfused region[22]. In practice, FFR derives from pressure measurements upstream and downstream of a given coronary stenosis at maximal

pharmacological arteriolar vasodilation. FFR was proposed as the best available technique to guide clinical decision making (whether mechanical revascularization or medical therapy), and FFR-guided PCI is the way to improved prognoses with respect to PCI based on angiographic severity[23-26].

Absolute CFR equals the ratio of maximal to baseline flow for any given arterial distribution with or without a stenosis or diffuse narrowing[27]. Relative CFR equals the ratio of maximal stress flow in the diseased artery to maximal flow in non-diseased arterial segments either in the same or adjacent arterial distribution. CFR in healthy volunteers with no risk factors is up to 4.5 ± 0.7. Values for mild disease in patients with coronary risk factors average 2.7 ± 0.6[28]. CFR < 2.0 is the proposed threshold for inducible ischemia[29]. FFR pressure ratio for a single discrete stenosis in the absence of diffuse disease also equals relative CFR using flow or flow velocity measurements. However, flow-based CFR and pressure-based FFR may not give evidence of comparable stenosis severity in approximately 40% of lesions[30]. Diffuse narrowing may reduce CFR significantly, with only a minimal fall in segmental pressure gradients or FFR. In situations of mixed diffuse and segmental disease, which are common in the clinical setting, noninvasive absolute maximal perfusion and CFR together likely define the severity of each coronary stenosis. Gould[31] provides the following example: "A 38% diffuse diameter narrowing in the absence of arterial remodeling would reduce CFR to 1.4 whereas the same diffuse disease plus a discrete angiographic 60% stenosis without remodeling would reduce CFR to 1.0, both without significant fall in FFR". Therefore, FFR is not a direct measure of low-flow ischemia, and it does not reflect absolute flow or absolute CFR, which are the determinants of ischemia. Further, FFR and CFR results may be inconsistent because microvascular resistance during hyperemia causes FFR and CFR to change in opposite directions. Higher hyperemic resistance means reduced maximal flow and higher distal pressure, *i.e.*, reduced CFR and increased FFR. Vice versa, low hyperemic microvascular resistance for equal stenosis means higher maximal flow and increased pressure gradient through the stenosis, which may result in low FFR.

Therefore, the microvascular compartment must be considered in an integrated view of coronary perfusion because its functional anatomy is complex. The branching of vessels in the myocardial wall, the different structure of subendocardium and subepicardium vessel wall, and the incremental vascular capacity across the myocardial wall depth describe this complexity, which is the substrate for more important functional differences that are physiologically intended to compensate the effects of myocardial contraction on subendocardial perfusion[32]. More intensely in early systole, the development of intraventricular pressure squeezes intramural vessels and reduces intramural blood volume, which causes coronary venous flow to increase and arterial flow to decrease[33].

The diagnosis of coronary microvascular disease likely occurs when CFR is significantly reduced in the absence of segmental perfusion defects. Coronary microvascular endothelial dysfunction may be assessed noninvasively by evaluating CFR with perfusion imaging in combination with cold pressor stress or demonstrating improvements in resting perfusion heterogeneity using a vasodilator stress. These methods may help correctly evaluate the physiological meaning of anatomical coronary stenosis when FFR and CFR results are inconclusive.

## PHARMACOLOGICAL STRESS TESTING

Two possible pharmacological methods of challenging coronary circulation evolved over the years: (1) pharmacological interaction with adenosine receptors of vascular smooth muscle cells (dipyridamole, adenosine, and regadenoson[34,35]); and (2) pharmacological inotropic stimulation (dobutamine[36]).

Adenosine vasodilator stress induces maximal increases in blood flow (three- to five-fold in healthy subjects) by reducing vascular resistance. The hyperemic flow is dependent on systemic pressure and the residual resistance at the microcirculation level *via* relaxation of smooth muscle cells, which uncouples coronary flow (supply) and myocardial work (demand). The mismatch between coronary flow and myocardial demand may induce the stealing phenomenon[34]. However, hyperemia induced by adenosine is mediated by endothelium receptors and neuronal-mediated mechanisms, which was elegantly demonstrated in two studies using L-nitroarginine methyl ester (L-NAME). Intracoronary L-NAME attenuated the hyperemic dilation in healthy volunteers *via* inhibition of endothelial nitric oxide synthase[37], but systemic L-NAME counterbalanced CFR increase *via* the neuronal response[38]. This effect may explain the limited accuracy of the ratio of hyperemic to rest MBF that exists in pathological conditions, such as hypertension, which is characterized by elevated flow at rest.

Dobutamine stressor is an alternative to physical stress. This synthetic sympathomimetic amine stimulates β- and α-adrenoreceptors, increases myocardial oxygen consumption (inotropic effect) and MBF[39-41]. Thickening of subendocardial myocardial layers primarily contributes to resting wall thickening, but catecholamines also stimulate a thickening of subepicardial layers. This effect is useful to detect myocardial viability, and it explains why patients with resting wall motion (WM) abnormalities consequent to subendocardial infarction still exhibit improved contractility during dobutamine infusion. This pharmacological effect is beneficial for the diagnosis of viability, but it may mask the subendocardial ischemia in patients with normal resting WM and significant coronary stenosis[42].

The cold pressor test deserves some attention for its capacity to interact with the sympathetic system[43-45].

Sympathetic stimulation using cold exposure induces a sharp rise in heart rate and systolic arterial pressure

and a norepinephrine release in the coronary circulation from adrenergic nerve terminals. This increase in rate-pressure product induces a similar increase in MBF, and norepinephrine vasoconstriction is balanced by endothelium-related vasodilation of epicardial coronary vessels and myocardial microcirculation. This mechanism is related to flow augmentation, and it exhibits a similar extent as pharmacologically induced hyperemia.

Therefore, the cold pressor test produces a similar increase in rate-pressure product and MBF. The effects on sympathetic endothelium modulation and endothelium-related flow augmentation suggest the utility of this stressor in evaluating microvascular function.

## CORONARY ARTERY STENOSIS DETECTION

The ischemic cascade concept, whereby abnormal perfusion precedes abnormal mechanical function during increased demand-induced ischemia, was demonstrated in an experimental setting. The spatial extent of perfusion abnormality is greater than the contraction abnormality, and the mismatch between perfusion and function is more evident in single-vessel *vs* multi-vessel disease[46].

Pharmacological and physical stress echocardiography is a mainstay of the noninvasive assessment of CAD. Technological advances and the use of contrast agents provided echocardiography the ability to increase and match the perfusion information to the classic evaluation of WM abnormalities that are induced by adrenergic or adenosinic mechanisms[47].

The use of echocardiographic contrast agents improved the accuracy of stress echocardiography[48-50]. Shah *et al*[48] demonstrated the usefulness of myocardial contrast stress echocardiography in 193 patients (88%) in a prospective clinical study, and it provided an incremental benefit over WM analysis in 25% of patients and greater confidence with WM evaluations in 62% of patients.

Thomas *et al*[51] randomized 1776 patients to real-time myocardial contrast (RTMCE) dobutamine, physical stress echocardiography or standard stress echocardiography in which contrast was used for opacification of the left ventricle only (non-RTMCE). Myocardial perfusion tests exhibited a higher positivity (22% for RTMCE *vs* 15% with non-RTMCE, $P = 0.0002$) with similar positive values to predict > 50% diameter stenosis using quantitative coronary angiography (67% for non-RTMCE, 73% for RTMCE). The higher positivity of RTMCE was related to the detection of subendocardial wall thickening abnormalities that were missed in non-RTMCE studies that only examined transmural wall thickening[51] (see the representative case in Figure 6).

At variance with a previous study[51], a large multicenter study[7] demonstrated a higher sensitivity of myocardial contrast stress echocardiography *vs* SPECT (75.2% *vs* 49.1%, $P < 0.0001$) for the detection of $\geqslant$ 70% or $\geqslant$ 50% stenosis, but the specificity was lower (52.4% *vs* 80.6%, $P < 0.0001$). Sensitivity for the detection of $\geqslant$ 70% single-vessel stenosis was higher for MCE (72.5% *vs* 42.7%, $P < 0.0001$) and the detection of proximal vessel disease (80% *vs* 58%, $P = 0.005$), but a cautionary note must be placed relative to fair inter-reader agreement ($k = 0.37$ for MCE, 0.34 for SPECT in the mentioned study)[15]. The sensitivity of MCE was greater for the detection of LAD and multivessel disease[15].

Dobutamine-atropine stress RTMCE and CMR exhibited comparable diagnostic accuracies for significant CAD detection due to the incremental values of myocardial perfusion imaging over WM analysis for RTMCE and CMR[52].

Myocardial contrast stress echocardiography also facilitates the measurement of the CFR-LAD using transthoracic Doppler, and CFR-LAD exhibited incremental value for WM analysis[53-55].

The prognostication potential of myocardial contrast stress echocardiography requires further research. MCE detects stress-induced perfusion defects that are uncoupled to WM abnormalities and identifies a sub-group of patients who are at a higher risk of coronary events among patients without inducible WM abnormalities at stress echocardiography, who are by definition at low risk[49,56-60].

Porter *et al*[60] studied 2014 patients with intermediate to high pre-test probability of CAD who were randomized to dobutamine or exercise stress RTMCE or conventional stress echocardiography (CSE) and followed prospectively for a median of 2.6 years. They demonstrated that patients with abnormal RTMCE studies had higher death rates, nonfatal myocardial infarction (MI), or subsequent revascularization rates than patients with abnormal CSE studies. No difference emerged in primary end-point rates following normal CSE or RTMCE studies. Notably, patients with perfusion defects and WM abnormalities and patients with perfusion defects only exhibited similar rates death/nonfatal MI (7.2% and 6.5%, respectively)[60].

Gaibazzi *et al*[61] confirmed these data in 718 patients in a multicenter cohort study who were followed for 16 mo after high-dose dipyridamole MCE with measurements of LAD flow reserve, and patients who underwent revascularization after the diagnostic test were censored[61].

## NO REFLOW

The "no reflow" phenomenon, first described by Ito *et al*[62] and subsequently confirmed by several investigators in MCE studies[63-67], refers to the situation when myocardial tissue perfusion is not restored despite a grade 3 TIMI flow on coronary angiography after primary coronary intervention for acute MI. The no reflow phenomenon influences the eventual infarct size and affects up to one third of patients who undergo reperfusion coronary interventions. This phenomenon

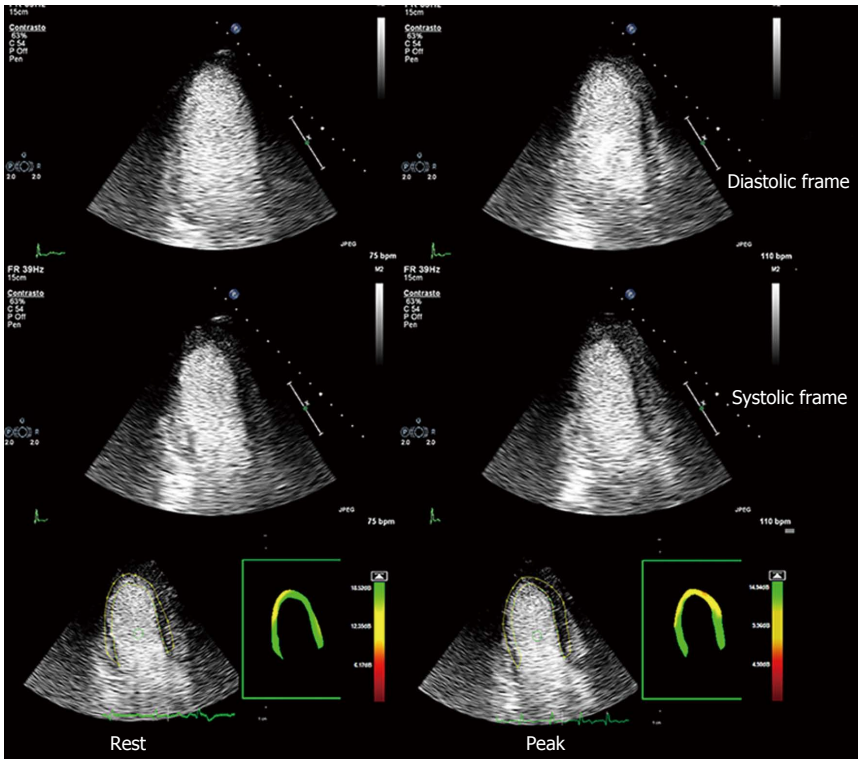


**Figure 6 Myocardial contrast stress echocardiography.** Apical three-chamber view of a patient with previous by-pass graft (left internal mammary artery graft onto left anterior descending coronary artery). Baseline diastolic and systolic frames on the left, top and intermediate rows, respectively; peak stress diastolic and systolic frames on the right, top and intermediate rows, respectively. Baseline and peak stress myocardial perfusion parametric quantification are displayed, respectively, in the bottom left and right panels. At baseline, akinesis of the infero-apical region is evident, which is concordant with a transmural defect of perfusion of the same region. At peak stress, no new wall motion abnormalities are detected, whereas parametric quantification of myocardial perfusion shows a large transmural defect of perfusion of all the apical regions and a subendocardial defect of perfusion of middle and basal anterior septum. A critical stenosis of distal mammary graft anastomosis was found on coronary angiography.

results from microvascular obstruction caused by the embolization of thrombus and plaque debris during balloon angioplasty and stent deployment in the setting of short duration myocardial ischemia (< 45 min). Other mechanisms are major determinants in prolonged ischemia, in which the no reflow correlates to microvascular damage. There may be areas of low reflow all around the no reflow area, whose salvage may be the potential target of treatment options. The presence of collaterals and the dynamics of the target vessel occlusion, whether it was an abrupt event or a chronic intermittent reduction of flow, influence the low reflow area.

Addressing the no reflow phenomenon may affect therapeutic intervention outcome in acute coronary syndromes. Several studies demonstrated that thrombus aspirations and occlusive protection devices may increase microvascular perfusion, and platelet inhibitors may reduce the no reflow area and infarct size. Pharmacological interventions (nicorandil, verapamil, adenosine) may also increase the chance to reduce myocardial infarct size[68-71].

## ISOLATED MICROVASCULAR DYSFUNCTION

Abnormalities of coronary microcirculation beyond myocardial ischemia caused by atherosclerosis of the epicardial coronary arteries are an alternative cause of, or may contribute to, myocardial ischemia in several conditions. Coronary microvascular disease (MVD) is a unique cause of symptoms in several patients with angina. This condition is known as microvascular angina (MVA)[72], and it is better defined as primary MVA to distinguish it from secondary MVA, which occurs in the setting of specific diseases (Table 1)[73].

The clinical presentation of MVA covers the entire spectrum of coronary syndromes, from chronic to unstable angina and acute syndromes.

Stable primary MVA is characterized by angina episodes that are exclusively or predominantly related to effort, and it can be identified with the clinical entity that is generally known as cardiac syndrome X[74]. No cardiac or systemic diseases should be detectable by definition. However, patients with uncomplicated hypertension or diabetes mellitus are often classified as syndrome X patients because these pathological conditions confer a risk for MVD similar to atherosclerosis obstructive CAD[74].

Functional abnormalities of resistive coronary vessels were documented in numerous studies on MVD. Blunted endothelium-dependent vasodilation due to impaired nitric oxide release is the most commonly proposed mechanism for MVD in stable MVA patients,

**Table 1 Pathogenetic classification of cardiac microvascular dysfunction**

| |
|---|
| MVD in the absence of myocardial and obstructive coronary artery diseases |
| MVD in the presence of myocardial disease |
| MVD in the presence of obstructive coronary artery disease |
| MVD caused by coronary recanalization interventions |

MVD: Microvascular dysfunction.

and it is based on a reduced coronary blow flow (CBF) response to acetylcholine[75]. A reduced CBF response to endothelium-independent vasodilators, such as adenosine, dipyridamole, and papaverine, was repeatedly reported[76-78], which suggests an important role of primary impaired relaxation of small vessels. Other studies demonstrated enhanced vasoconstrictor activity in coronary microcirculation in several patients with stable MVA. Ergonovine injection, mental stress, and hyperventilation resulted in impairments of CBF[79]. Tests to diagnose MVD in the clinical setting should explore the vasodilation and vasoconstriction responses of coronary microcirculation. Vasodilator tests are the first choice in patients with stable MVA, but the response to vasoconstrictor stimuli should be assessed when the former tests are normal or inconclusive. Transthoracic Doppler echocardiographic evaluation of CBF may be used as a first-line method to identify MVD in the LAD territory of patients with normal coronary arteries with suspected MVA. Contrast stress echocardiography may represent the frontier method to detect MVD in the entire myocardial circulation[80].

Unstable primary MVA should be suspected in patients with non-ST-segment elevation acute coronary syndrome and normal coronary arteries on angiography. *De novo* abnormalities on standard ECG in these patients (*e.g.*, ST-segment depression, negative T waves), their gradual normalization, and mild elevation of serum markers of myocardial damage (troponins) indicate a cardiac ischemic origin of symptoms. Diagnosis requires the exclusion of epicardial coronary spasm and transient coronary thrombosis as the cause of angina together with evidence of MVD.

Multiple studies evaluated the prognosis in MVD and demonstrated more cardiac events in patients with reduced CFR. However, there is no consensus in the literature on the best prognostic CFR cutoff (range 1.5-2.5). Murthy *et al*[81] demonstrated a 5.6-fold increased risk of cardiac death in patients with suspected CAD and CFR < 1.5.

Two forms of unstable MVA are described, microvascular variant angina and stress-related cardiomyopathy. Mohri *et al*[82] described the first form in Japanese patients with angina attacks at rest (less frequently with associated effort angina) in the presence of normal coronary arteries. Intracoronary acetylcholine reproduced angina and ST-segment changes in these patients. The absence of vasospasm of epicardial coronary vessels suggested diffuse coronary microvascular spasm.

Stress-related cardiomyopathy (also known as apical ballooning syndrome or takotsubo disease) is generally triggered by sudden emotional or even physically intense stress[83]. Acute chest pain may be associated with abrupt heart failure or cardiogenic shock. The clinical picture includes normal epicardial coronary arteries, depressed left ventricular function, left ventricular ballooning at angiography or echocardiography due to apical and mid-ventricular akinesia with preserved contraction of basal segments, relatively minor elevations of troponins and creatine kinase-MB and a favorable clinical course with recovery of all abnormalities in 1 to 3 mo. The disease is considered adrenergic-mediated because of the cause-effect relationship with stress. Findings that support this hypothesis include increased catecholamine levels, histological signs of catecholamine-mediated cardiotoxicity in endomyocardial biopsy specimens[84], and the unique distribution of cardiac WM abnormalities, which may reflect the variable distribution of adrenergic innervation in the myocardium[84]. Sustained intense coronary microvascular constriction or spasm induced by excessive adrenergic stimulation that results in myocardial ischemia and stunning may be an alternative pathological mechanism, at least in some patients[85]. Some reports demonstrated abnormal myocardial perfusion in the affected myocardial segments[86], and MVD was documented by evidence of reduced CBF responses to vasodilator stimuli in the acute phase[87]. MVD subsided in several weeks, which paralleled clinical improvement[87], but subclinical microvascular dysfunction persisted long after the acute phase. This persistence was demonstrated by the abnormal response of coronary flow to the cold pressor test and left ventricular regional contraction on contrast echocardiography[88]. Our follow-up study of patients with takotsubo syndrome demonstrated transient WM abnormalities and reduced CFR without regional myocardial perfusion abnormalities in response to the cold pressor test, which suggests the persistence of microvascular dysfunction (Figure 7).

## CONCLUSION

Second-generation echocardiographic contrast agents received regulatory authorities' approval for clinical use in left ventricular opacification studies with the only limitation of patients with intracardiac shunts or pulmonary hypertension. In contrast, MCE did not receive approval for clinical use, and the technique remains an option for research. MCE is a demanding technique in the technical skills that are required of sonographers and physicians and the investment of software of analysis systems. The reimbursement issue may represent an adjunctive drawback. Reimbursement for MCE studies is approximately $60 in the United States, but reimbursement covers only the cost of the drug in most European countries. Analysis of cost/effectiveness and cost saving based on the procedural

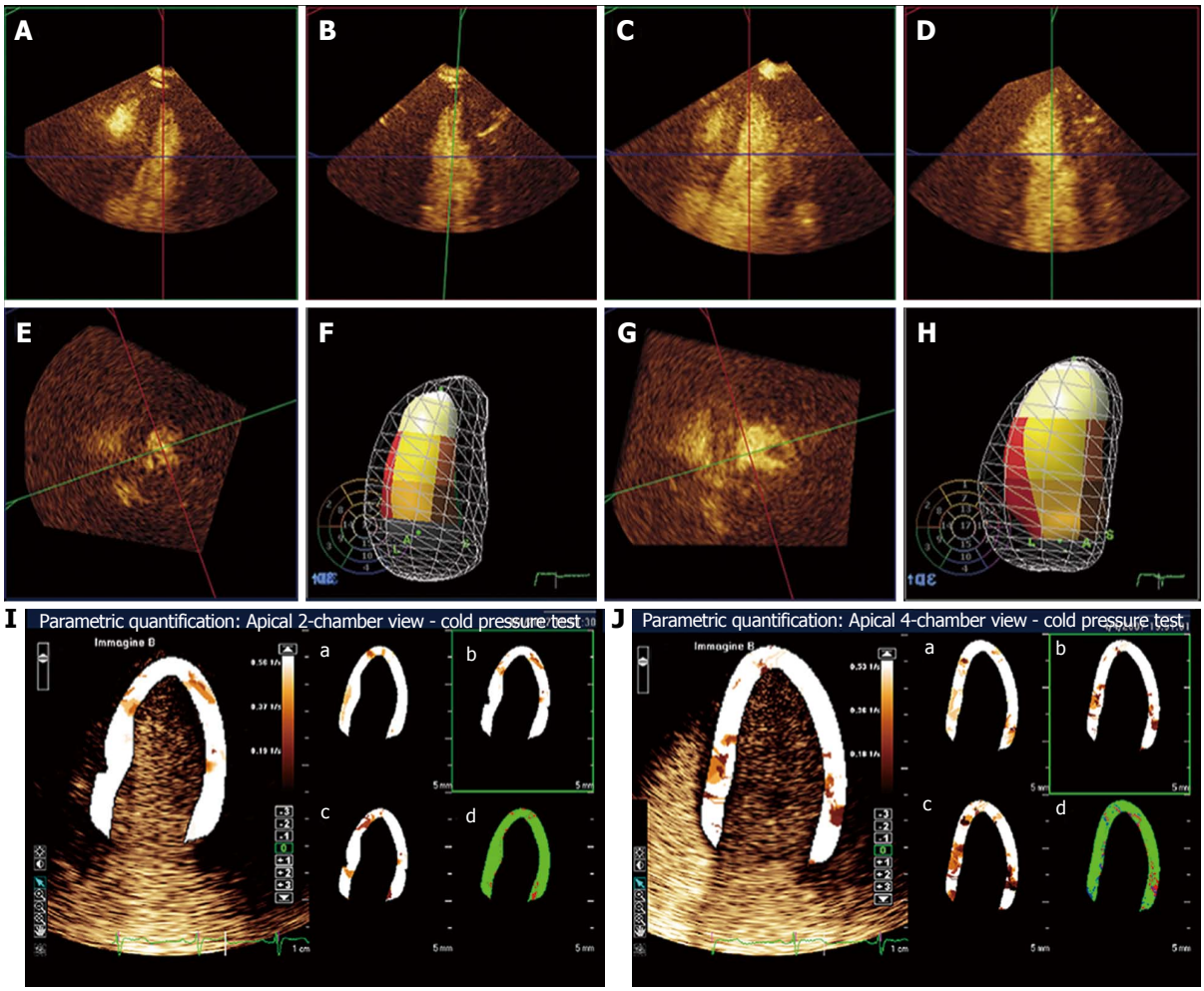


**Figure 7 Real-time 3-dimensional myocardial contrast echocardiography during follow-up using cold pressor test in a patient who recovered from apical ballooning syndrome.** A, B and E: Reconstructed 4-chamber (A), 2-chamber (B) and short-axis (E) end-systolic frames at baseline; F: 3-dimensional systolic volume rendering as left ventricular cast inside the diastolic mesh volume rendering. The American Society of Echocardiography 17-segment model of the left ventricle is reproduced as a bulls-eye in the background, and superimposed color-coded onto the left ventricular cast; C, D and G: Reconstructed 4-chamber (C), 2-chamber (D) and short-axis (G) end-systolic frames using the cold pressor test; H: The diastolic and systolic 3-dimensional casts as in panel 1d. Note that wall motion is normal at baseline, whereas apical akinesia develops during the cold pressor test; I and J: Apical 2-chamber and 4-chamber, respectively, parametric myocardial contrast echocardiography quantification using the cold pressor test; the slope β of the replenishment curve is superimposed onto the left ventricular wall in 2-chamber and 4-chamber views, respectively. Perfusion parameters A, β and A × β are superimposed onto the same left ventricular wall as in panels I and J, respectively, in panels a, b and c; panels d represents the goodness of fit. Parametric images demonstrate homogeneous perfusion during the cold pressor test. The coronary flow reserve in this patient was 1.10 (normal range 2.77 ± 0.70).

and downstream investigation costs, as the one conducted by the Medical Services Advisory Committee of Australia on the use of second-generation contrast agents in patients with suboptimal echocardiograms[89], is lacking for MCE. However, the diagnostic potentials of MCE, which covers the entire spectrum of cardiac circulation physiopathology and clinical presentation of coronary syndromes, may reduce overall costs.

Research has established that the application fields for MCE represent the clinical perspective to pursue this technique in the future daily workflow of echo laboratories. The integration of FFR with quantitative MCE offers the opportunity to demonstrate the effects of anatomical diffuse non-critical coronary stenosis and furnishes an integrated vision of the extent of ischemic burden that is comprehensive of collateral flow contribution. MCE in the setting of acute coronary syndromes identifies complex anatomo-functional features, such as no reflow and low flow, within and around the infarct area, respectively, which foresees the potential for functional recovery of stunned myocardium to guide therapeutic interventions. In an era when typical angina is less frequently associated with significant coronary stenosis[90] and acute coronary syndromes are dissociated from intracoronary thrombosis or significant coronary stenosis in up to 10% of cases, MCE is a relatively low-expensive, bedside technique to examine microvascular reserve and identify patients with MVD.

In conclusion, current research provides good evidence that MCE improves comprehensive echocardiographic evaluations of ischemic heart disease. The approval of regulatory authorities and the availability of quantitative operator-independent analysis software will hopefully prompt physicians and sonographers to

implement MCE into the daily work flow of echo laboratories.

## REFERENCES


1 **Diamond GA**, Forrester JS. Analysis of probability as an aid in the clinical diagnosis of coronary-artery disease. *N Engl J Med* 1979; **300**: 1350-1358 [PMID: 440357 DOI: 10.1056/NEJM197906143002402]

2 **Appleby MA**, Angeja BG, Dauterman K, Gibson CM. Angiographic assessment of myocardial perfusion: TIMI myocardial perfusion (TMP) grading system. *Heart* 2001; **86**: 485-486 [PMID: 11602533 DOI: 10.1136/heart.86.5.485]

3 **Kakuta K**, Dohi K, Yamada T, Yamanaka T, Kawamura M, Nakamori S, Nakajima H, Tanigawa T, Onishi K, Yamada N, Nakamura M, Ito M. Detection of coronary artery disease using coronary flow velocity reserve by transthoracic Doppler echocardiography versus multidetector computed tomography coronary angiography: influence of calcium score. *J Am Soc Echocardiogr* 2014; **27**: 775-785 [PMID: 24679739 DOI: 10.1016/j.echo.2014.02.012]

4 **Jaarsma C**, Leiner T, Bekkers SC, Crijns HJ, Wildberger JE, Nagel E, Nelemans PJ, Schalla S. Diagnostic performance of noninvasive myocardial perfusion imaging using single-photon emission computed tomography, cardiac magnetic resonance, and positron emission tomography imaging for the detection of obstructive coronary artery disease: a meta-analysis. *J Am Coll Cardiol* 2012; **59**: 1719-1728 [PMID: 22554604 DOI: 10.1016/j.jacc.2011.12.040]

5 **Wolk MJ**, Bailey SR, Doherty JU, Douglas PS, Hendel RC, Kramer CM, Min JK, Patel MR, Rosenbaum L, Shaw LJ, Stainback RF, Allen JM. ACCF/AHA/ASE/ASNC/HFSA/HRS/SCAI/SCCT/SCMR/STS 2013 multimodality appropriate use criteria for the detection and risk assessment of stable ischemic heart disease: a report of the American College of Cardiology Foundation Appropriate Use Criteria Task Force, American Heart Association, American Society of Echocardiography, American Society of Nuclear Cardiology, Heart Failure Society of America, Heart Rhythm Society, Society for Cardiovascular Angiography and Interventions, Society of Cardiovascular Computed Tomography, Society for Cardiovascular Magnetic Resonance, and Society of Thoracic Surgeons. *J Am Coll Cardiol* 2014; **63**: 380-406 [PMID: 24355759 DOI: 10.1016/j.jacc.2013.11.009]

6 **de Jong MC**, Genders TS, van Geuns RJ, Moelker A, Hunink MG. Diagnostic performance of stress myocardial perfusion imaging for coronary artery disease: a systematic review and meta-analysis. *Eur Radiol* 2012; **22**: 1881-1895 [PMID: 22527375 DOI: 10.1007/s00330-012-2434-1]

7 **Senior R**, Moreo A, Gaibazzi N, Agati L, Tiemann K, Shivalkar B, von Bardeleben S, Galiuto L, Lardoux H, Trocino G, Carrió I, Le Guludec D, Sambuceti G, Becher H, Colonna P, Ten Cate F, Bramucci E, Cohen A, Bezante G, Aggeli C, Kasprzak JD. Comparison of sulfur hexafluoride microbubble (SonoVue)-enhanced myocardial contrast echocardiography with gated single-photon emission computed tomography for detection of significant coronary artery disease: a large European multicenter study. *J Am Coll Cardiol* 2013; **62**: 1353-1361 [PMID: 23770168 DOI: 10.1016/j.jacc.2013.04.082]

8 **Hsu B**, Chen FC, Wu TC, Huang WS, Hou PN, Chen CC, Hung GU. Quantitation of myocardial blood flow and myocardial flow reserve with 99mTc-sestamibi dynamic SPECT/CT to enhance detection of coronary artery disease. *Eur J Nucl Med Mol Imaging* 2014; **41**: 2294-2306 [PMID: 25143072 DOI: 10.1007/s00259-014-2881-9]

9 **Ben-Haim S**, Murthy VL, Breault C, Allie R, Sitek A, Roth N, Fantony J, Moore SC, Park MA, Kijewski M, Haroon A, Slomka P, Erlandsson K, Baavour R, Zilberstien Y, Bomanji J, Di Carli MF. Quantification of Myocardial Perfusion Reserve Using Dynamic SPECT Imaging in Humans: A Feasibility Study. *J Nucl Med* 2013; **54**: 873-879 [PMID: 23578996 DOI: 10.2967/jnumed.112.109652]

10 **Shiraishi S**, Sakamoto F, Tsuda N, Yoshida M, Tomiguchi S, Utsunomiya D, Ogawa H, Yamashita Y. Prediction of left main or 3-vessel disease using myocardial perfusion reserve on dynamic thallium-201 single-photon emission computed tomography with a semiconductor gamma camera. *Circ J* 2015; **79**: 623-631 [PMID: 25746547 DOI: 10.1253/circj.CJ-14-0932]

11 **Cerqueira MD**, Allman KC, Ficaro EP, Hansen CL, Nichols KJ, Thompson RC, Van Decker WA, Yakovlevitch M. Recommendations for reducing radiation exposure in myocardial perfusion imaging. *J Nucl Cardiol* 2010; **17**: 709-718 [PMID: 20503120 DOI: 10.1007/s12350-010-9244-0]

12 **Schindler TH**, Schelbert HR, Quercioli A, Dilsizian V. Cardiac PET imaging for the detection and monitoring of coronary artery disease and microvascular health. *JACC Cardiovasc Imaging* 2010; **3**: 623-640 [PMID: 20541718 DOI: 10.1016/j.jcmg.2010.04.007]

13 **Morton G**, Chiribiri A, Ishida M, Hussain ST, Schuster A, Indermuehle A, Perera D, Knuuti J, Baker S, Hedström E, Schleyer P, O'Doherty M, Barrington S, Nagel E. Quantification of absolute myocardial perfusion in patients with coronary artery disease: comparison between cardiovascular magnetic resonance and positron emission tomography. *J Am Coll Cardiol* 2012; **60**: 1546-1555 [PMID: 22999722 DOI: 10.1016/j.jacc.2012.05.052]

14 **Biglands JD**, Magee DR, Sourbron SP, Plein S, Greenwood JP, Radjenovic A. Comparison of the Diagnostic Performance of Four Quantitative Myocardial Perfusion Estimation Methods Used in Cardiac MR Imaging: CE-MARC Substudy. *Radiology* 2015; **275**: 393-402 [PMID: 25521666 DOI: 10.1148/radiol.14140433]

15 **Thomas JD**. Myocardial contrast echocardiography perfusion imaging: still waiting after all these years. *J Am Coll Cardiol* 2013; **62**: 1362-1364 [PMID: 23770171 DOI: 10.1016/j.jacc.2013.05.053]

16 **Vogel R**, Indermühle A, Reinhardt J, Meier P, Siegrist PT, Namdar M, Kaufmann PA, Seiler C. The quantification of absolute myocardial perfusion in humans by contrast echocardiography: algorithm and validation. *J Am Coll Cardiol* 2005; **45**: 754-762 [PMID: 15734622 DOI: 10.1016/j.jacc.2004.11.044]

17 **Yu EH**, Skyba DM, Leong-Poi H, Sloggett C, Jamorski M, Garg R, Iwanochko RM, Siu SC. Incremental value of parametric quantitative assessment of myocardial perfusion by triggered Low-Power myocardial contrast echocardiography. *J Am Coll Cardiol* 2004; **43**: 1807-1813 [PMID: 15145104 DOI: 10.1016/j.jacc.2003.09.073]

18 **Tune JD**, Gorman MW, Feigl EO. Matching coronary blood flow to myocardial oxygen consumption. *J Appl Physiol* (1985) 2004; **97**: 404-415 [PMID: 15220323 DOI: 10.1152/japplphysiol.01345.2003]

19 **Mosher P**, Ross J, Mcfate PA, Shaw RF. Control of coronary blood flow by an autoregulatory mechanism. *Circ Res* 1964; **14**: 250-259 [PMID: 14133952 DOI: 10.1161/01.RES.14.3.250]

20 **White CW**, Wright CB, Doty DB, Hiratza LF, Eastham CL, Harrison DG, Marcus ML. Does visual interpretation of the coronary arteriogram predict the physiologic importance of a coronary stenosis? *N Engl J Med* 1984; **310**: 819-824 [PMID: 6700670 DOI: 10.1056/NEJM198403293101304]

21 **Meijboom WB**, Van Mieghem CA, van Pelt N, Weustink A, Pugliese F, Mollet NR, Boersma E, Regar E, van Geuns RJ, de Jaegere PJ, Serruys PW, Krestin GP, de Feyter PJ. Comprehensive assessment of coronary artery stenoses: computed tomography coronary angiography versus conventional coronary angiography and correlation with fractional flow reserve in patients with stable angina. *J Am Coll Cardiol* 2008; **52**: 636-643 [PMID: 18702967 DOI: 10.1016/j.jacc.2008.05.024]

22 **Pijls NH**, van Son JA, Kirkeeide RL, De Bruyne B, Gould KL. Experimental basis of determining maximum coronary, myocardial, and collateral blood flow by pressure measurements for assessing functional stenosis severity before and after percutaneous transluminal coronary angioplasty. *Circulation* 1993; **87**: 1354-1367 [PMID: 8462157 DOI: 10.1161/01.CIR.87.4.1354]

23 **Zhang D**, Lv S, Song X, Yuan F, Xu F, Zhang M, Yan S, Cao X. Fractional flow reserve versus angiography for guiding percu-

taneous coronary intervention: a meta-analysis. *Heart* 2015; **101**: 455-462 [PMID: 25637372 DOI: 10.1136/heartjnl-2014-306578]
24 **Pijls NH**, Fearon WF, Tonino PA, Siebert U, Ikeno F, Bornschein B, van't Veer M, Klauss V, Manoharan G, Engstrøm T, Oldroyd KG, Ver Lee PN, MacCarthy PA, De Bruyne B. Fractional flow reserve versus angiography for guiding percutaneous coronary intervention in patients with multivessel coronary artery disease: 2-year follow-up of the FAME (Fractional Flow Reserve Versus Angiography for Multivessel Evaluation) study. *J Am Coll Cardiol* 2010; **56**: 177-184 [PMID: 20537493 DOI: 10.1016/j.jacc.2010.04.012]
25 **Wijns W**, Kolh P, Danchin N, Di Mario C, Falk V, Folliguet T, Garg S, Huber K, James S, Knuuti J, Lopez-Sendon J, Marco J, Menicanti L, Ostojic M, Piepoli MF, Pirlet C, Pomar JL, Reifart N, Ribichini FL, Schalij MJ, Sergeant P, Serruys PW, Silber S, Sousa Uva M, Taggart D. Guidelines on myocardial revascularization. *Eur Heart J* 2010; **31**: 2501-2555 [PMID: 20802248 DOI: 10.1093/eurheartj/ehq277]
26 **Levine GN**, Bates ER, Blankenship JC, Bailey SR, Bittl JA, Cercek B, Chambers CE, Ellis SG, Guyton RA, Hollenberg SM, Khot UN, Lange RA, Mauri L, Mehran R, Moussa ID, Mukherjee D, Nallamothu BK, Ting HH. 2011 ACCF/AHA/SCAI Guideline for Percutaneous Coronary Intervention. A report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines and the Society for Cardiovascular Angiography and Interventions. *J Am Coll Cardiol* 2011; **58**: e44-122 [PMID: 22070834 DOI: 10.1016/j.jacc.2011.08.007]
27 **Hoffman JI**. Maximal coronary flow and the concept of coronary vascular reserve. *Circulation* 1984; **70**: 153-159 [PMID: 6234109 DOI: 10.1161/01.CIR.70.2.153]
28 **Kern MJ**, Bach RG, Mechem CJ, Caracciolo EA, Aguirre FV, Miller LW, Donohue TJ. Variations in normal coronary vasodilatory reserve stratified by artery, gender, heart transplantation and coronary artery disease. *J Am Coll Cardiol* 1996; **28**: 1154-1160 [PMID: 8890809 DOI: 10.1016/S0735-1097(96)00327-0]
29 **Kern MJ**, Lerman A, Bech JW, De Bruyne B, Eeckhout E, Fearon WF, Higano ST, Lim MJ, Meuwissen M, Piek JJ, Pijls NH, Siebes M, Spaan JA. Physiological assessment of coronary artery disease in the cardiac catheterization laboratory: a scientific statement from the American Heart Association Committee on Diagnostic and Interventional Cardiac Catheterization, Council on Clinical Cardiology. *Circulation* 2006; **114**: 1321-1341 [PMID: 16940193 DOI: 10.1161/CIRCULATIONAHA.106.177276]
30 **van de Hoef TP**, van Lavieren MA, Damman P, Delewi R, Piek MA, Chamuleau SA, Voskuil M, Henriques JP, Koch KT, de Winter RJ, Spaan JA, Siebes M, Tijssen JG, Meuwissen M, Piek JJ. Physiological basis and long-term clinical outcome of discordance between fractional flow reserve and coronary flow velocity reserve in coronary stenoses of intermediate severity. *Circ Cardiovasc Interv* 2014; **7**: 301-311 [PMID: 24782198 DOI: 10.1161/CIRCINTERVENTIONS.113.001049]
31 **Gould KL**. Does coronary flow trump coronary anatomy? *JACC Cardiovasc Imaging* 2009; **2**: 1009-1023 [PMID: 19679290 DOI: 10.1016/j.jcmg.2009.06.004]
32 **Chilian WM**. Microvascular pressures and resistances in the left ventricular subepicardium and subendocardium. *Circ Res* 1991; **69**: 561-570 [PMID: 1873859 DOI: 10.1161/01.RES.69.3.561]
33 **Spaan JA**. Mechanical determinants of myocardial perfusion. *Basic Res Cardiol* 1995; **90**: 89-102 [PMID: 7646422 DOI: 10.1007/BF00789439]
34 **Picano E**. Stress echocardiography. From pathophysiological toy to diagnostic tool. *Circulation* 1992; **85**: 1604-1612 [PMID: 1555297 DOI: 10.1161/01.CIR.85.4.1604]
35 **Palani G**, Ananthasubramaniam K. Regadenoson: review of its established role in myocardial perfusion imaging and emerging applications. *Cardiol Rev* 2013; **21**: 42-48 [PMID: 22643345 DOI: 10.1097/CRD.0b013e3182613db6]
36 **Geleijnse ML**, Elhendy A, Fioretti PM, Roelandt JR. Dobutamine stress myocardial perfusion imaging. *J Am Coll Cardiol* 2000; **36**: 2017-2027 [PMID: 11127435 DOI: 10.1016/S0735-1097(00)01012-3]
37 **Quyyumi AA**, Dakak N, Andrews NP, Husain S, Arora S, Gilligan DM, Panza JA, Cannon RO. Nitric oxide activity in the human coronary circulation. Impact of risk factors for coronary atherosclerosis. *J Clin Invest* 1995; **95**: 1747-1755 [PMID: 7706483 DOI: 10.1172/JCI117852]
38 **Kaufmann PA**, Rimoldi O, Gnecchi-Ruscone T, Bonser RS, Lüscher TF, Camici PG. Systemic inhibition of nitric oxide synthase unmasks neural constraint of maximal myocardial blood flow in humans. *Circulation* 2004; **110**: 1431-1436 [PMID: 15353503 DOI: 10.1161/01.CIR.0000141294.25130.54]
39 **Krivokapich J**, Huang SC, Schelbert HR. Assessment of the effects of dobutamine on myocardial blood flow and oxidative metabolism in normal human subjects using nitrogen-13 ammonia and carbon-11 acetate. *Am J Cardiol* 1993; **71**: 1351-1356 [PMID: 8498380 DOI: 10.1016/0002-9149(93)90554-P]
40 **Severi S**, Underwood R, Mohiaddin RH, Boyd H, Paterni M, Camici PG. Dobutamine stress: effects on regional myocardial blood flow and wall motion. *J Am Coll Cardiol* 1995; **26**: 1187-1195 [PMID: 7594031 DOI: 10.1016/0735-1097(95)00319-3]
41 **Skopicki HA**, Abraham SA, Picard MH, Alpert NM, Fischman AJ, Gewirtz H. Effects of dobutamine at maximally tolerated dose on myocardial blood flow in humans with ischemic heart disease. *Circulation* 1997; **96**: 3346-3352 [PMID: 9396426 DOI: 10.1161/01.CIR.96.10.3346]
42 **Xie F**, Dodla S, O'Leary E, Porter TR. Detection of subendocardial ischemia in the left anterior descending coronary artery territory with real-time myocardial contrast echocardiography during dobutamine stress echocardiography. *JACC Cardiovasc Imaging* 2008; **1**: 271-278 [PMID: 19356438 DOI: 10.1016/j.jcmg.2008.02.004]
43 **Nabel EG**, Ganz P, Gordon JB, Alexander RW, Selwyn AP. Dilation of normal and constriction of atherosclerotic coronary arteries caused by the cold pressor test. *Circulation* 1988; **77**: 43-52 [PMID: 2826047 DOI: 10.1161/01.CIR.77.1.43]
44 **Sanderson JE**, Woo KS, Chung HK, Chan WM, Tse KK, White HD. Endothelium-dependent dilation of the coronary arteries in syndrome X: effects of the cold pressor test. *Cardiology* 1997; **88**: 414-417 [PMID: 9286502 DOI: 10.1159/000177370]
45 **Pham I**, Nguyen MT, Valensi P, Rousseau H, Nitenberg A, Vicaut E, Cosson E. Noninvasive study of coronary microcirculation response to a cold pressor test. *Eur J Clin Invest* 2015; **45**: 135-143 [PMID: 25490913 DOI: 10.1111/eci.12389]
46 **Leong-Poi H**, Rim SJ, Le DE, Fisher NG, Wei K, Kaul S. Perfusion versus function: the ischemic cascade in demand ischemia: implications of single-vessel versus multivessel stenosis. *Circulation* 2002; **105**: 987-992 [PMID: 11864930 DOI: 10.1161/hc0802.104326]
47 **Sicari R**, Nihoyannopoulos P, Evangelista A, Kasprzak J, Lancellotti P, Poldermans D, Voigt JU, Zamorano JL. Stress echocardiography expert consensus statement: European Association of Echocardiography (EAE) (a registered branch of the ESC). *Eur J Echocardiogr* 2008; **9**: 415-437 [PMID: 18579481 DOI: 10.1093/ejechocard/jen175]
48 **Shah BN**, Chahal NS, Bhattacharyya S, Li W, Roussin I, Khattar RS, Senior R. The feasibility and clinical utility of myocardial contrast echocardiography in clinical practice: results from the incorporation of myocardial perfusion assessment into clinical testing with stress echocardiography study. *J Am Soc Echocardiogr* 2014; **27**: 520-530 [PMID: 24637056 DOI: 10.1016/j.echo.2014.01.028]
49 **Plana JC**, Mikati IA, Dokainish H, Lakkis N, Abukhalil J, Davis R, Hetzell BC, Zoghbi WA. A randomized cross-over study for evaluation of the effect of image optimization with contrast on the diagnostic accuracy of dobutamine echocardiography in coronary artery disease The OPTIMIZE Trial. *JACC Cardiovasc Imaging* 2008; **1**: 145-152 [PMID: 19356420 DOI: 10.1016/j.jcmg.2007.10.014]
50 **Jeetley P**, Hickman M, Kamp O, Lang RM, Thomas JD, Vannan MA, Vanoverschelde JL, van der Wouw PA, Senior R. Myocardial

contrast echocardiography for the detection of coronary artery stenosis: a prospective multicenter study in comparison with single-photon emission computed tomography. *J Am Coll Cardiol* 2006; **47**: 141-145 [PMID: 16386678 DOI: 10.1016/j.jacc.2005.08.054]

51 **Thomas D**, Xie F, Smith LM, O'Leary E, Smith K, Olson J, Nalty K, Hess R, Graham M, Therrien S, Porter TR. Prospective randomized comparison of conventional stress echocardiography and real-time perfusion stress echocardiography in detecting significant coronary artery disease. *J Am Soc Echocardiogr* 2012; **25**: 1207-1214 [PMID: 22998856 DOI: 10.1016/j.echo.2012.08.016]

52 **Falcão SN**, Rochitte CE, Junior WM, Quaglia L, Lemos PA, Sbano JC, Ramires JA, Kalil Filho R, Tsutsui JM. Incremental value of perfusion over wall-motion abnormalities with the use of dobutamine-atropine stress myocardial contrast echocardiography and magnetic resonance imaging for detecting coronary artery disease. *Echocardiography* 2013; **30**: 45-54 [PMID: 23006451 DOI: 10.1111/j.1540-8175.2012.01820.x]

53 **Cortigiani L**, Rigo F, Gherardi S, Sicari R, Galderisi M, Bovenzi F, Picano E. Additional prognostic value of coronary flow reserve in diabetic and nondiabetic patients with negative dipyridamole stress echocardiography by wall motion criteria. *J Am Coll Cardiol* 2007; **50**: 1354-1361 [PMID: 17903635 DOI: 10.1016/j.jacc.2007.06.027]

54 **Rigo F**, Sicari R, Gherardi S, Djordjevic-Dikic A, Cortigiani L, Picano E. The additive prognostic value of wall motion abnormalities and coronary flow reserve during dipyridamole stress echo. *Eur Heart J* 2008; **29**: 79-88 [PMID: 18063595 DOI: 10.1093/eurheartj/ehm527]

55 **Cortigiani L**, Rigo F, Gherardi S, Galderisi M, Bovenzi F, Picano E, Sicari R. Prognostic effect of coronary flow reserve in women versus men with chest pain syndrome and normal dipyridamole stress echocardiography. *Am J Cardiol* 2010; **106**: 1703-1708 [PMID: 21126613 DOI: 10.1016/j.amjcard.2010.08.011]

56 **Tsutsui JM**, Elhendy A, Anderson JR, Xie F, McGrain AC, Porter TR. Prognostic value of dobutamine stress myocardial contrast perfusion echocardiography. *Circulation* 2005; **112**: 1444-1450 [PMID: 16129798 DOI: 10.1161/CIRCULATIONAHA.105.537134]

57 **Tsutsui JM**, Xie F, Cloutier D, Kalvaitis S, Elhendy A, Porter TR. Real-time dobutamine stress myocardial perfusion echocardiography predicts outcome in the elderly. *Eur Heart J* 2008; **29**: 377-385 [PMID: 17989076 DOI: 10.1093/eurheartj/ehm445]

58 **Gaibazzi N**, Squeri A, Reverberi C, Molinaro S, Lorenzoni V, Sartorio D, Senior R. Contrast stress-echocardiography predicts cardiac events in patients with suspected acute coronary syndrome but nondiagnostic electrocardiogram and normal 12-hour troponin. *J Am Soc Echocardiogr* 2011; **24**: 1333-1341 [PMID: 22014426 DOI: 10.1016/j.echo.2011.09.002]

59 **Dawson D**, Kaul S, Peters D, Rinkevich D, Schnell G, Belcik JT, Wei K. Prognostic value of dipyridamole stress myocardial contrast echocardiography: comparison with single photon emission computed tomography. *J Am Soc Echocardiogr* 2009; **22**: 954-960 [PMID: 19553084 DOI: 10.1016/j.echo.2009.04.034]

60 **Porter TR**, Smith LM, Wu J, Thomas D, Haas JT, Mathers DH, Williams E, Olson J, Nalty K, Hess R, Therrien S, Xie F. Patient outcome following 2 different stress imaging approaches: a prospective randomized comparison. *J Am Coll Cardiol* 2013; **61**: 2446-2455 [PMID: 23643501 DOI: 10.1016/j.jacc.2013.04.019]

61 **Gaibazzi N**, Rigo F, Lorenzoni V, Molinaro S, Bartolomucci F, Reverberi C, Marwick TH. Comparative prediction of cardiac events by wall motion, wall motion plus coronary flow reserve, or myocardial perfusion analysis: a multicenter study of contrast stress echocardiography. *JACC Cardiovasc Imaging* 2013; **6**: 1-12 [PMID: 23219414 DOI: 10.1016/j.jcmg.2012.08.009]

62 **Ito H**, Tomooka T, Sakai N, Yu H, Higashino Y, Fujii K, Masuyama T, Kitabatake A, Minamino T. Lack of myocardial perfusion immediately after successful thrombolysis. A predictor of poor recovery of left ventricular function in anterior myocardial infarction. *Circulation* 1992; **85**: 1699-1705 [PMID: 1572028 DOI: 10.1161/01.CIR.85.5.1699]

63 **Ragosta M**, Camarano G, Kaul S, Powers ER, Sarembock IJ, Gimple LW. Microvascular integrity indicates myocellular viability in patients with recent myocardial infarction. New insights using myocardial contrast echocardiography. *Circulation* 1994; **89**: 2562-2569 [PMID: 8205665 DOI: 10.1161/01.CIR.89.6.2562]

64 **Lepper W**, Kamp O, Vanoverschelde JL, Franke A, Sieswerda GT, Pasquet A, Kühl HP, Voci P, Visser CA, Hanrath P, Hoffmann R. Intravenous myocardial contrast echocardiography predicts left ventricular remodeling in patients with acute myocardial infarction. *J Am Soc Echocardiogr* 2002; **15**: 849-856 [PMID: 12221399 DOI: 10.1067/mje.2002.121277]

65 **Janardhanan R**, Swinburn JM, Greaves K, Senior R. Usefulness of myocardial contrast echocardiography using low-power continuous imaging early after acute myocardial infarction to predict late functional left ventricular recovery. *Am J Cardiol* 2003; **92**: 493-497 [PMID: 12943865 DOI: 10.1016/S0002-9149(03)00713-6]

66 **Dwivedi G**, Janardhanan R, Hayat SA, Swinburn JM, Senior R. Prognostic value of myocardial viability detected by myocardial contrast echocardiography early after acute myocardial infarction. *J Am Coll Cardiol* 2007; **50**: 327-334 [PMID: 17659200 DOI: 10.1016/j.jacc.2007.03.036]

67 **Funaro S**, La Torre G, Madonna M, Galiuto L, Scarà A, Labbadia A, Canali E, Mattatelli A, Fedele F, Alessandrini F, Crea F, Agati L. Incidence, determinants, and prognostic value of reverse left ventricular remodelling after primary percutaneous coronary intervention: results of the Acute Myocardial Infarction Contrast Imaging (AMICI) multicenter study. *Eur Heart J* 2009; **30**: 566-575 [PMID: 19098019 DOI: 10.1093/eurheartj/ehn529]

68 **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**: 2154-2159 [PMID: 10801755 DOI: 10.1161/01.CIR.101.18.2154101.18.2154]

69 **Ross AM**, Gibbons RJ, Stone GW, Kloner RA, Alexander RW. A randomized, double-blinded, placebo-controlled multicenter trial of adenosine as an adjunct to reperfusion in the treatment of acute myocardial infarction (AMISTAD-II). *J Am Coll Cardiol* 2005; **45**: 1775-1780 [PMID: 15936605 DOI: 10.1016/j.jacc.2005.02.061]

70 **Ito H**, Taniyama Y, Iwakura K, Nishikawa N, Masuyama T, Kuzuya T, Hori M, Higashino Y, Fujii K, Minamino T. Intravenous nicorandil can preserve microvascular integrity and myocardial viability in patients with reperfused anterior wall myocardial infarction. *J Am Coll Cardiol* 1999; **33**: 654-660 [PMID: 10080465 DOI: 10.1016/S0735-1097(98)00604-4]

71 **Taniyama Y**, Ito H, Iwakura K, Masuyama T, Hori M, Takiuchi S, Nishikawa N, Higashino Y, Fujii K, Minamino T. Beneficial effect of intracoronary verapamil on microvascular and myocardial salvage in patients with acute myocardial infarction. *J Am Coll Cardiol* 1997; **30**: 1193-1199 [PMID: 9350914 DOI: 10.1016/S0735-1097(97)00277-5]

72 **Cannon RO**, Epstein SE. "Microvascular angina" as a cause of chest pain with angiographically normal coronary arteries. *Am J Cardiol* 1988; **61**: 1338-1343 [PMID: 3287885 DOI: 10.1016/0002-9149(88)91180-0]

73 **Camici PG**, Crea F. Coronary microvascular dysfunction. *N Engl J Med* 2007; **356**: 830-840 [PMID: 17314342 DOI: 10.1056/NEJMra061889]

74 **Lanza GA**. Cardiac syndrome X: a critical overview and future perspectives. *Heart* 2007; **93**: 159-166 [PMID: 16399854 DOI: 10.1136/hrt.2005.067330]

75 **Bøttcher M**, Botker HE, Sonne H, Nielsen TT, Czernin J. Endothelium-dependent and -independent perfusion reserve and the effect of L-arginine on myocardial perfusion in patients with syndrome X. *Circulation* 1999; **99**: 1795-1801 [PMID: 10199874 DOI: 10.1161/01.CIR.99.14.179599.14.1795]

76 **Opherk D**, Zebe H, Weihe E, Mall G, Dürr C, Gravert B, Mehmel HC, Schwarz F, Kübler W. Reduced coronary dilatory capacity and ultrastructural changes of the myocardium in patients with angina pectoris but normal coronary arteriograms. *Circulation* 1981; **63**: 817-825 [PMID: 7471337 DOI: 10.1161/01.CIR.63.4.817]

77 **Motz W**, Vogt M, Rabenau O, Scheler S, Lückhoff A, Strauer BE.

Evidence of endothelial dysfunction in coronary resistance vessels in patients with angina pectoris and normal coronary angiograms. *Am J Cardiol* 1991; **68**: 996-1003 [PMID: 1927940 DOI: 10.1016/0002-9149(91)90485-4]

78 **Panting JR**, Gatehouse PD, Yang GZ, Grothues F, Firmin DN, Collins P, Pennell DJ. Abnormal subendocardial perfusion in cardiac syndrome X detected by cardiovascular magnetic resonance imaging. *N Engl J Med* 2002; **346**: 1948-1953 [PMID: 12075055 DOI: 10.1056/NEJMoa012369]

79 **Chauhan A**, Mullins PA, Taylor G, Petch MC, Schofield PM. Effect of hyperventilation and mental stress on coronary blood flow in syndrome X. *Br Heart J* 1993; **69**: 516-524 [PMID: 8343318 DOI: 10.1136/hrt.69.6.516]

80 **Galiuto L**, Sestito A, Barchetta S, Sgueglia GA, Infusino F, La Rosa C, Lanza G, Crea F. Noninvasive evaluation of flow reserve in the left anterior descending coronary artery in patients with cardiac syndrome X. *Am J Cardiol* 2007; **99**: 1378-1383 [PMID: 17493464 DOI: 10.1016/j.amjcard.2006.12.070]

81 **Murthy VL**, Naya M, Foster CR, Hainer J, Gaber M, Di Carli G, Blankstein R, Dorbala S, Sitek A, Pencina MJ, Di Carli MF. Improved cardiac risk assessment with noninvasive measures of coronary flow reserve. *Circulation* 2011; **124**: 2215-2224 [PMID: 22007073 DOI: 10.1161/CIRCULATIONAHA.111.050427]

82 **Mohri M**, Koyanagi M, Egashira K, Tagawa H, Ichiki T, Shimokawa H, Takeshita A. Angina pectoris caused by coronary microvascular spasm. *Lancet* 1998; **351**: 1165-1169 [PMID: 9643687 DOI: 10.1016/S0140-6736(97)07329-7]

83 **Bybee KA**, Prasad A. Stress-related cardiomyopathy syndromes. *Circulation* 2008; **118**: 397-409 [PMID: 18645066 DOI: 10.1161/CIRCULATIONAHA.106.677625]

84 **Wittstein IS**, Thiemann DR, Lima JA, Baughman KL, Schulman SP, Gerstenblith G, Wu KC, Rade JJ, Bivalacqua TJ, Champion HC. Neurohumoral features of myocardial stunning due to sudden emotional stress. *N Engl J Med* 2005; **352**: 539-548 [PMID: 15703419 DOI: 10.1056/NEJMoa043046]

85 **Baumgart D**, Heusch G. Neuronal control of coronary blood flow. *Basic Res Cardiol* 1995; **90**: 142-159 [PMID: 7646417 DOI: 10.1007/BF00789444]

86 **Kurisu S**, Inoue I, Kawagoe T, Ishihara M, Shimatani Y, Nishioka K, Umemura T, Nakamura S, Yoshida M, Sato H. Myocardial perfusion and fatty acid metabolism in patients with tako-tsubo-like left ventricular dysfunction. *J Am Coll Cardiol* 2003; **41**: 743-748 [PMID: 12628716 DOI: 10.1016/S0735-1097(02)02924-8]

87 **Meimoun P**, Malaquin D, Sayah S, Benali T, Luycx-Bore A, Levy F, Zemir H, Tribouilloy C. The coronary flow reserve is transiently impaired in tako-tsubo cardiomyopathy: a prospective study using serial Doppler transthoracic echocardiography. *J Am Soc Echocardiogr* 2008; **21**: 72-77 [PMID: 17628401 DOI: 10.1016/j.echo.2007.05.024]

88 **Barletta G**, Del Pace S, Boddi M, Del Bene R, Salvadori C, Bellandi B, Coppo M, Saletti E, Gensini GF. Abnormal coronary reserve and left ventricular wall motion during cold pressor test in patients with previous left ventricular ballooning syndrome. *Eur Heart J* 2009; **30**: 3007-3014 [PMID: 19700469 DOI: 10.1093/eurheartj/ehp325]

89 **Thomson K**, Atherton J, Platts D, Lott CW, Savage R, Rimmer S; Australia Medical Services Advisory Committee. Second-generation contrast agents for use in patients with suboptimal echocardiograms. Canberra ACT, Australia: Medical Services Advisory Committee, 2010: MSAC application 1129. Available from: URL: http://www.msac.gov.au/internet/msac/publishing.nsf/Content/BAE45713D7D0FDEBCA257817001CB46D

90 **Genders TS**, Steyerberg EW, Alkadhi H, Leschka S, Desbiolles L, Nieman K, Galema TW, Meijboom WB, Mollet NR, de Feyter PJ, Cademartiri F, Maffei E, Dewey M, Zimmermann E, Laule M, Pugliese F, Barbagallo R, Sinitsyn V, Bogaert J, Goetschalckx K, Schoepf UJ, Rowe GW, Schuijf JD, Bax JJ, de Graaf FR, Knuuti J, Kajander S, van Mieghem CA, Meijs MF, Cramer MJ, Gopalan D, Feuchtner G, Friedrich G, Krestin GP, Hunink MG. A clinical prediction rule for the diagnosis of coronary artery disease: validation, updating, and extension. *Eur Heart J* 2011; **32**: 1316-1330 [PMID: 21367834 DOI: 10.1093/eurheartj/ehr014]

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