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SPOTLIGHT REVIEW

ESC Working Group on Coronary Pathophysiology and Microcirculation position paper on 'coronary microvascular dysfunction in cardiovascular disease'

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Although myocardial ischaemia usually manifests as a consequence of atherosclerosis-dependent obstructive epicardial coronary artery disease, a significant percentage of patients suffer ischaemic events in the absence of epicardial coronary artery obstruction. Experimental and clinical evidence highlight the abnormalities of the coronary microcirculation as a main cause of myocardial ischaemia in patients with 'normal or near normal' coronary arteries on angiography. Coronary microvascular disturbances have been associated with early stages of atherosclerosis even prior to any angiographic evidence of epicardial coronary stenosis, as well as to other cardiac pathologies such as myocardial hypertrophy and heart failure. The main objectives of the manuscript are (i) to provide updated evidence in our current understanding of the pathophysiological consequences of microvascular dysfunction in the heart; (ii) to report on the current knowledge on the relevance of cardiovascular risk factors and comorbid conditions for microcirculatory dysfunction; and (iii) to evidence the relevance of the clinical consequences of microvascular dysfunction. Highlighting the clinical importance of coronary microvascular dysfunction will open the field for research and the development of novel strategies for intervention will encourage early detection of subclinical disease and will help in the stratification of cardiovascular risk in agreement with the new concept of precision medicine.

Keywords Microvessels • Coronary microcirculation • Molecular and cellular targets • Risk factors • Ischaemic heart disease

This article is part of the Spotlight Issue on Coronary Microvascular Dysfunction.

1. Introduction

Myocardial infarction (MI) and myocardial ischaemia have been traditionally considered a 'large vessel ' disease caused by atherosclerosis and obstructive atherothrombotic events in epicardial coronary arteries that lead to the complete occlusion of the vessel at the 'culprit site '. While the coronary microcirculation is not affected by atherosclerosis, impairment of microvascular blood flow is an established cause of myocardial ischaemia.

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Coronary microcirculation refers to different anatomically and functionally vascular compartments (<500 μm diameter) with a critical role in the physiological regulation of myocardial perfusion. Coronary microcirculation is responsible for the regulation of blood flow distribution to cover the metabolic demands of the myocardium and the modulation of peripheral vascular resistance. In healthy individuals, without significant epicardial atherosclerosis, increases in myocardial metabolic demand are met by progressive vasodilation of coronary arterioles, which can normally induce a five-fold increase of coronary blood flow.

Coronary microvascular dysfunction (CMD) refers to a term covering a wide spectrum of clinical situations in which the structure and function of the coronary microcirculation is affected. This leads to impaired responses of the coronary flow to vasodilator stimuli, being characterized by impaired coronary flow reserve (CFR) with cut-off values below 2.0–2.5 depending on the methodology, or abnormal high index of coronary microvascular resistance (IMR; e.g. IMR >25) and/or focal or diffuse vasoconstriction during acetylcholine provocation testing, in the absence of any significant epicardial coronary artery obstruction (>50% lumen stenosis at coronary angiography) or preserved fractional flow reserve (FFR, value ≥ 0.80).[1,2]

A wide spectrum of cardiac and systemic conditions can lead to myocardial ischaemia due to CMD without significant epicardial coronary obstruction[1,3,4] These diseases are mainly those that cause left ventricular hypertrophy (hypertrophic cardiomyopathy, aortic stenosis, and hypertensive heart disease) or inflammation (myocarditis or vasculitis).[1,3–6] However, often, it is in the absence of other diseases that functional and/or structural alterations in coronary microcirculation are responsible for myocardial ischaemia. This condition affects up to 50% of subjects with chronic coronary syndromes, up to 20% of those with acute coronary syndromes (ACS) and associates with an increase in event rates.

In light of these considerations, the objective of the present position paper of the European Society of Cardiology (ESC) Working Group on Coronary Pathophysiology and Microcirculation is to provide updated evidence and a critical view in our understanding of the pathophysiology of microvascular dysfunction in association with ischaemic heart disease. This includes a detailed discussion, based on clinical and experimental evidence, on the relevance of cardiovascular risk factors and comorbid conditions in CMD, and a critical view on the relevance of CMD in non-obstructive ischaemic heart disease and its clinical consequences in ST-segment elevation myocardial infarction (STEMI) patients.

2. Coronary microcirculation and ischaemic heart disease

CMD is a multifactorial disease, prevalent in a wide spectrum of clinical cardiovascular situations. Traditionally, CMD has been classified according to the clinical setting in which it may occur.[5] Here, CMD is discussed in relation to the phenotype and severity of coronary artery disease (CAD) (non-obstructive chronic coronary syndrome, obstructive chronic coronary syndrome, non-obstructive ACS, obstructive ACS, and no-reflow), highlighting aspects from pathophysiology to treatment, including diagnosis, incidence, and prognosis.

2.1 CMD in chronic coronary syndrome

2.1.1 CMD in non-obstructive chronic coronary syndrome

A significant number of patients with symptoms and signs of ischaemic heart disease do not have a significant coronary artery stenosis (>50%).[6–8] This phenomenon termed 'ischaemia with non-obstructive coronary artery disease (INOCA)' is associated with increased risk for major cardiovascular events compared with an asymptomatic reference population.[9,10]

Major risk factors for INOCA include dyslipidaemia, obesity, metabolic syndrome, and diabetes.[11] The pathophysiological link between metabolic dysregulation and INOCA most likely involves CMD (*Figure 1*). The mechanisms underlying CMD in non-obstructive CAD are still not completely understood, but they implicate both functional and structural changes. Thus, both experimental[12,13] and clinical[14] studies have shown that endothelial dysfunction is a key mediator in the pathogenesis of CMD. This includes attenuation of endothelium-dependent vasodilation resulting from reduced nitric oxide (NO) bioavailability[12] and increased vasoconstrictor responses to endothelin-1 (ET-1), prostaglandin H2, and thromboxane A_2,[13] in swine models of metabolic dysregulation.

Increased sympathetic activity produces exaggerated alpha-adrenergic coronary vasoconstriction in patients with metabolic syndrome.[15] Likewise, in patients with pre-hypertension and metabolic syndrome, activation of the renin– angiotensin–aldosterone system increases angiotensin II-mediated vasoconstriction in the coronary circulation.[16] In addition, adipocyte-derived free fatty acids and leptin also lead to increased adrenergic tone.[16] Experimental studies have shown that adipocytes and perivascular adipose tissue-derived adipokines such as leptin, resistin, IL-6, and tumour necrosis factor-α are potent pro-inflammatory molecules that can promote oxidative stress in the endothelium and impair endothelial function and NO bioavailability, either directly or via increased ET-1 production.[11,17]

Changes in microvascular structure also contribute to CMD. Ossabaw swine with MetS had a blunted response to adenosine along with a reduced microvascular density.[18] In a similar model, hyperaemic flow was impaired with hypertrophic inward remodelling of coronary resistance arteries, capillary rarefaction, and augmented myogenic tone in isolated coronary arterioles.[19] Taken together, microvascular dysfunction, inward remodelling of resistance arteries and reductions in vascular density can all reduce flow reserve and produce regional ischaemia in the absence of an epicardial stenosis in humans and in animal models with comorbidities.[11,17]

2.1.2 CMD in obstructive chronic coronary syndrome

Differing from the healthy heart, in which the coronary microcirculation is the principal site of vascular resistance, in the setting of a physiologically significant epicardial artery stenosis, this additional resistance limits maximal coronary blood flow. While some of this reduction is secondary to the pressure loss across the stenosis , [20] there is also evidence that abnormalities in the control of coronary microvascular tone and microvascular structure, can contribute to the reductions in CFR.[21] Experimental studies have demonstrated that decreases in post-stenotic perfusion pressure can trigger structural and functional alterations in the distal microvasculature.[20] These include inward remodelling of the coronary resistance arteries and coronary arteriolar and capillary rarefaction distal to a coronary stenosis. Capillary rarefaction is also seen in dysfunctional myocardium of patients undergoing coronary bypass surgery and is a predictor of poor functional recovery following revascularization.[22] The concept that such structural microvascular abnormalities persist long after revascularization is supported by experimental studies in swine, in which weeks after revascularization of hibernating myocardium, resting flow normalizes but the flow response to metabolic stress (i.e. high-dose

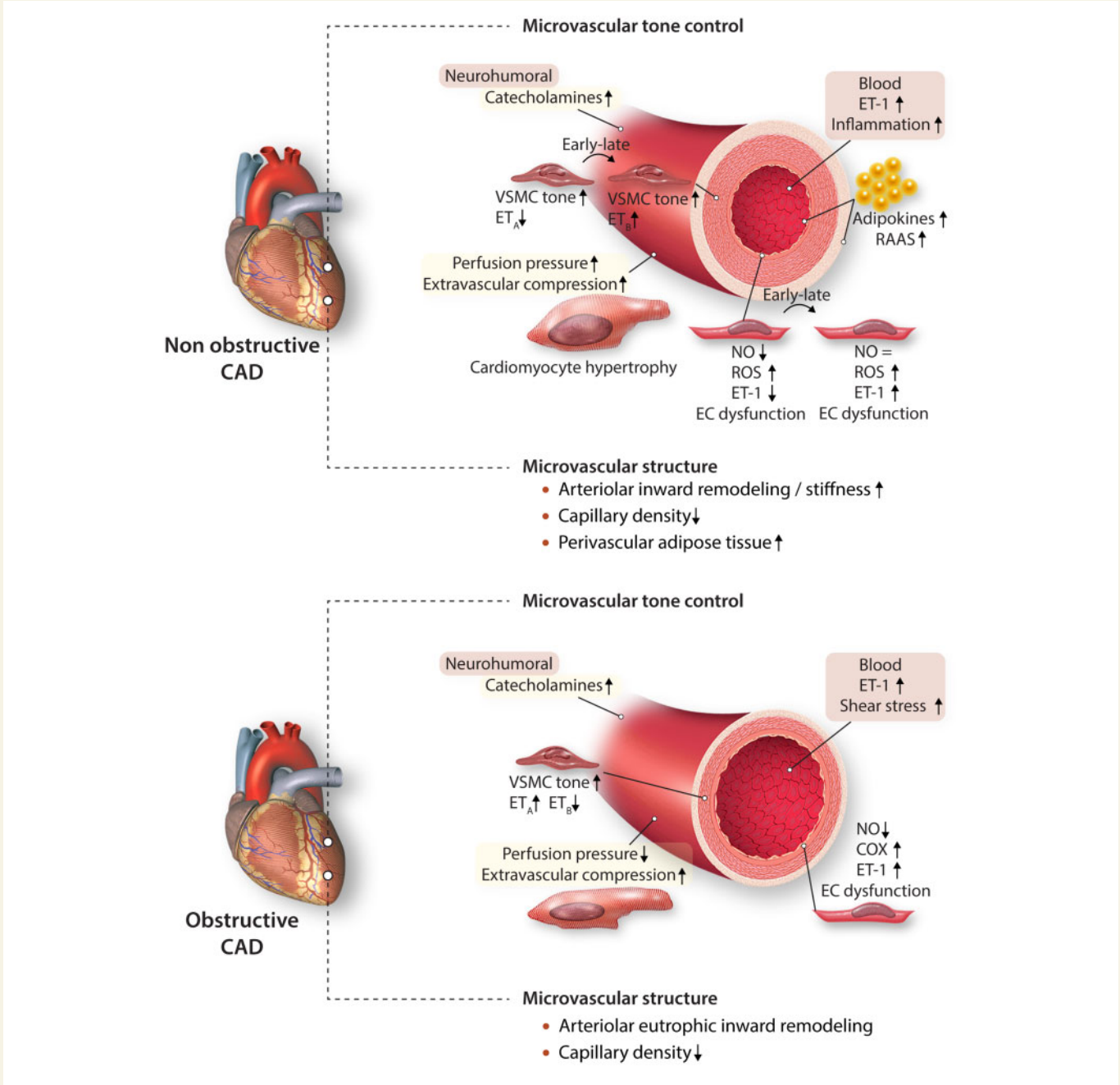

Figure 1 Coronary microvascular dysfunction in non-obstructive and obstructive coronary artery disease. COX, cyclooxygenase; EC, endothelial cell; ET-1, endothelin-1; ETA, endothelin receptor A; ETB, endothelin receptor B; NO, nitric oxide; RAAS, renin–angiotensin–aldosterone system; ROS, reactive oxygen species; VSMC, vascular smooth muscle cell.

dobutamine infusion) remains blunted.[23] Microvascular rarefaction occurs rather rapidly because it is already detected after 90 min left anterior descending artery occlusion in an experimental model of MI in swine and can be partially recovered by delivery of stem cells or stem cell-derived products.[24] This is an area of active research because it may represent a novel therapeutic intervention to reduce CMD.

Interestingly, the recently presented ISCHEMIA trial (International Study of Comparative Health Effectiveness With Medical and Invasive Approaches —recently presented at the American Heart Association - AHA- scientific session 2019) showed that revascularization was not better that optimal medical therapy, in stable patients with at least moderate ischaemia and ≥50% stenosis in a major epicardial vessel. In this very complicated and long trial, the reduction of the epicardial stenosis did not produce the expected benefits supporting the concept of the importance of mechanisms independent of epicardial arteries in ischaemia symptoms.

Functional coronary microvascular remodelling distal to a stenosis may reflect impaired vasodilator function as well as increased vasoconstrictor responses, as supported by experimental studies in dogs.[25] In this respect, exaggerated ET-1 induced vasoconstriction was documented in isolated coronary arterioles from swine with a chronic stenosis (*Figure 1*).[26] Surprisingly, vasodilator responses to bradykinin were preserved while ET_B-mediated vasodilation was lost. The preservation of

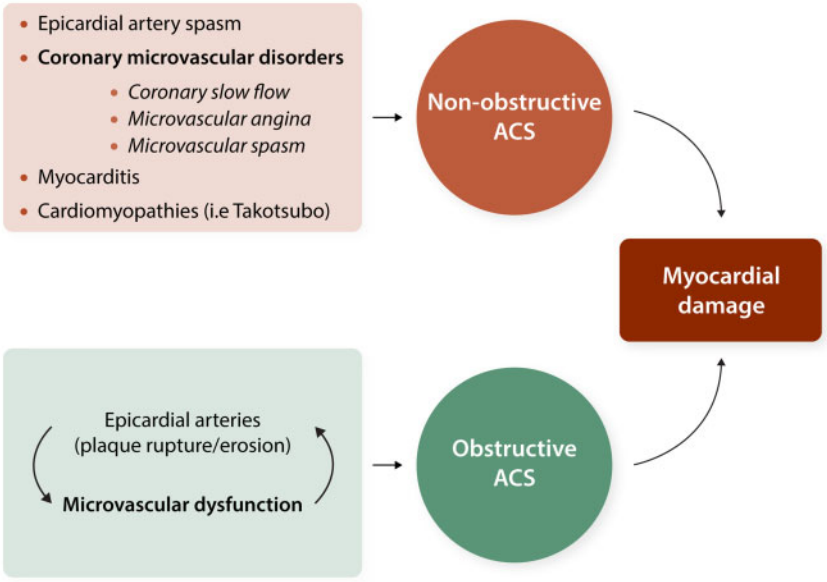

Figure 2 Microvascular dysfunction as underlying pathophysiological mechanism for acute coronary syndromes.

endothelial vasodilation to bradykinin may have reflected a shift in mechanism from NO to dilator endothelium-derived hyperpolarizing factor (EDHF). Indeed, the contribution of EDHF to the regulation of coronary microvascular tone in humans has been shown to accompany the loss of NO with the progression of CAD.[27] Experimental studies have shown that structural inward remodelling of arterioles can be produced at low intraluminal pressure *in vitro* and is enhanced by ET-1 and prevented by the calcium-antagonist amlodipine.[28]

2.2 CMD in ACS

2.2.1 CMD in non-obstructive ACS

ACS with normal or near-normal coronary arteries on coronary angiography refers to a heterogeneous clinical entity with multiple potential causes that not always are apparent. Within this condition, MINOCA is defined as MI with non-obstructed coronary arteries (< 50% diameter stenosis in a major epicardial vessel) that refers to an ischaemic mechanism responsible for myocyte injury and troponin elevation. The concept of MINOCA has been recently included in the STEMI guidelines (2017) of the European Society of Cardiology.[3] MINOCA is more common in women than in men and in patients presenting with non-STEMI (NSTEMI) than those presenting with STEMI.[29]

Pathophysiology of non-obstructive ACS may involve epicardial or microvascular causes (i.e. CMD) and cardiac non-ischaemic aetiologies (i.e. myocarditis or Takotsubo syndrome) (*Figure 2*).[5,30] Myocardial disorders, including the Takotsubo cardiomyopathy, were initially included into the definition of MINOCA.[31] However, according to the recently published Fourth Universal Definition of Myocardial Infarction, diagnosis of MINOCA, such as diagnosis of MI, refer to ischaemic mechanisms responsible for the myocyte injury, whereas non-ischaemic causes such as myocarditis have been excluded.[32] Among MINOCA patients, coronary microvascular spasm is suggested to account for 16% of cases.[33] Patients with non-obstructive ACS exhibit a significant coronary dysfunction, which seems to involve both an increased constrictor reactivity, likely mainly involving the coronary microcirculation, and a reduced microvascular dilator function both persisting at 12-month follow-up.[31,34]

2.2.2 CMD in obstructive ACS

It is generally assumed that epicardial events precede and are cause of microvascular dysfunction. However, a novel concept of the mechanisms resulting in ACS takes into account the primary dysfunction of the microcirculation in addition to the vulnerable plaque as causal contributor to myocardial damage and infarct size (*Figure 2*). Thus, transient or permanent microvascular dysfunction limits coronary blood flow and leads to alterations of the shear stress affecting endothelial function and enhancing thrombus formation at epicardial level.[35]

A major challenge for considering the primary role of CMD is the lack of data on microvascular function prior to an ACS. Thus, much work remains to understand the mechanisms involving microcirculatory impairment prior, during and after ACS.

2.2.3 CMD and coronary no-reflow

Timely reperfusion therapy after acute myocardial infarction (AMI) is a key factor in reducing infarct size and improving ventricular function and clinical outcome. Unfortunately, even in the absence of a residual epicardial stenosis, revascularization does not always re-establish effective microcirculatory perfusion due to the no-reflow phenomenon , [36] which occurs more frequently in women.[37] Results in animal models suggest that suboptimal reperfusion after percutaneous coronary intervention (PCI) is related to microvascular disorders resulting from microvascular obstruction (MVO) secondary to endothelial injury and/or distal embolization.[38] In addition, experimental studies define MVO as an independent predictor of adverse left ventricular remodelling.[39] Moreover, a recent pooled analysis of individual data from seven randomized trials supports the notion that presence and extent of MVO after primary PCI in STEMI patients strongly associates with major adverse cardiovascular events occurring within a year.[40]

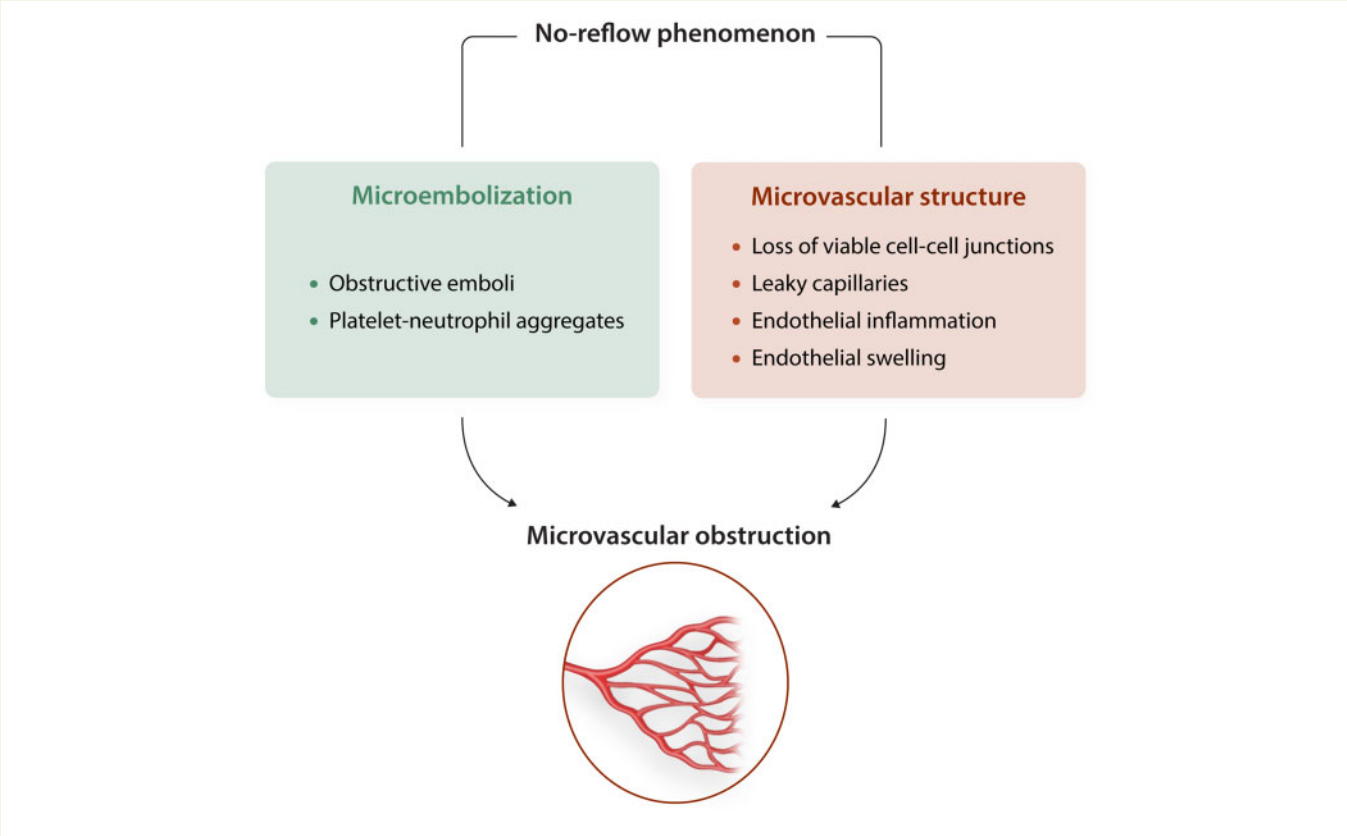

Figure 3 Pathophysiological mechanisms of microvascular dysfunction associated to the non-reflow phenomenon.

Coronary microvascular endothelial damage, resulting from either ischaemia–reperfusion and/or the associated comorbidities (metabolic dysfunction, hypertension, ageing, and dyslipidaemia), is thought to play a critical role in the no-reflow phenomenon.[41] However, other mechanisms have also been proposed to contribute, including activation of inflammatory pathways, myocyte oedema, platelet activation, or leucocyte infiltration.[38] Interruption of blood flow, followed by its acute restoration, results in endothelial dysfunction leading to alterations in the balance between the different vasomotor pathways, as summarized in *Figure 3*. With increasing severity of ischaemia–reperfusion injury, even more extensive microvascular injury occurs, with increased vascular permeability, thinning of the capillary wall and loss of viable cell–cell junctions, leading to oedema and even intramyocardial haemorrhage.[42] Although there is no evidence that no-reflow contributes to secondary cardiomyocyte damage, it may affect infarct healing responses —i.e. lead to infarct thinning —and may thus aggravate post-infarct remodelling.[43] Recent studies on P2Y12 inhibitors in a porcine model of ischaemia/reperfusion have shown a significant reduction in no-reflow in treated animals.[44,45] Novel preclinical and clinical studies are needed to reduce MVO and improve clinical outcomes.

2.3 CMD in reperfused acute MI

Moreover, there is clinical evidence showing that CMD may contribute to the persistence or recurrence of angina following successful revascularization after AMI.[46,47] This represents an important unresolved clinical problem that affects from one-fifth to one-third of patients undergoing myocardial revascularization at 1-year follow-up. CMD in reperfused AMI is associated with adverse remodelling, lower ventricular function, and worse prognostic.[48] Up to now, however, there is a recognized lack of knowledge of how ameliorate CMD and improve signs and symptoms of myocardial ischaemia in these patients, in part due to an insufficient understanding of the underlaying pathophysiology. Multiple underlying causes can contribute to CMD after successful PCI and stenting, from endothelial dysfunction, increased oxidative stress, reduced NO-release, to low shear stress conditions distal to the stenosis that may negatively influence microvascular function. In addition, a masked CMD pre-existing to PCI might also account for the prevalence of anginal symptoms and/or myocardial ischaemia after a successful PCI.[49] Signs of ischaemia/reperfusion injury affecting coronary microcirculation go from reversible interstitial oedema that acts compressing coronary microcirculation to capillary destruction with intramyocardial haemorrhage. Interstitial iron deposition induces an inflammatory response that contributes to microvascular injury.[50] Several mechanisms contributing to microvascular injury in reperfused AMI do not differ from those contributing to cardiomyocyte injury. To date, however, the temporal and causal relationship between both pathologic conditions needs to be clarified.[50]

3. Factors increasing the risk of CMD

Risk factors for CMD do not differ from those for epicardial macrovascular arterial disease including diabetes mellitus, obesity, hypertension, hyperlipidaemia, smoking, and age, which, separately and synergistically,

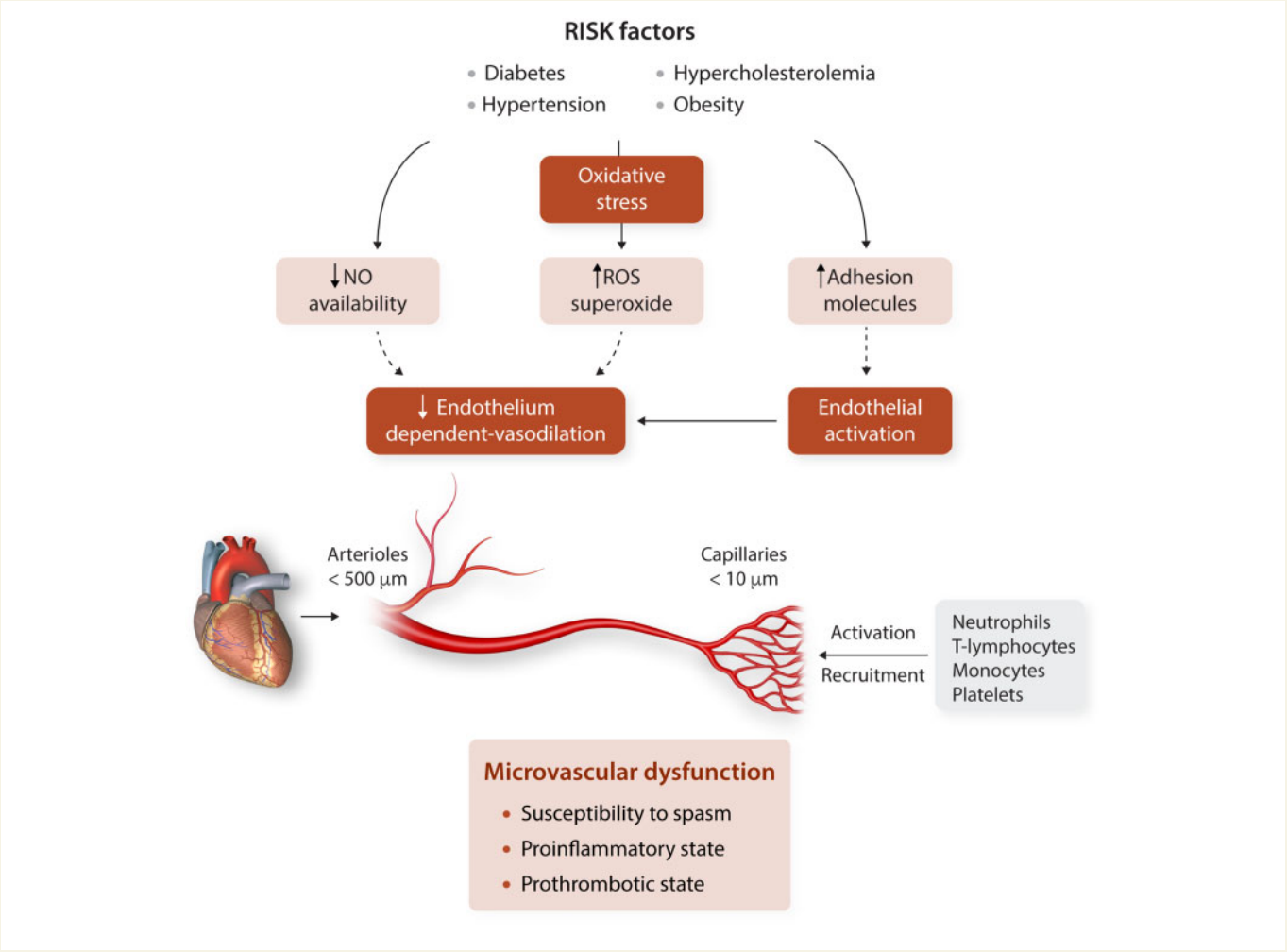

Figure 4 Microvascular response to risk factors. NO, nitric oxide; ROS, reactive oxygen species.

contribute to CMD (*Figure 4*). Thus, an increasing number of traditional cardiovascular risk factors associate with reduced values of myocardial perfusion reserve index and abnormal microvascular dilation leading to impaired CFR.

3.1 Diabetes and obesity

Diabetes is a strong inducer of microvascular dysfunction, but the relationship is bidirectional since microvascular dysfunction in muscle and adipose tissue also contributes to the pathogenesis of type 2 diabetes.[51] As CMD precedes hyperglycaemia in the pathogenesis of type 2 diabetes, factors other than hyperglycaemia contribute to CMD in type 2 diabetes. CMD in diabetes is characterized by decreased NO activity, increased production of reactive oxygen species (ROS), increased endothelin synthesis, reduced endothelial barrier function, and elevated inflammatory activity oxidative stress, a basic pathological mechanism in ischaemic myocardial injury, is caused by an imbalance between excessive production and detoxification of free radicals, especially ROS, in the vascular endothelial cells resulting in the oxidative modification of cellular and extracellular components directly affecting cell function and viability. Diabetes is an endocrine multiorgan disease, and consequently, diabetes-associated CMD has been detected in multiple organs including the heart, retina, kidney, and skin. In pigs, diabetes decreases myocardial blood flow and capillary density (rarefaction).[52]

Overweight and obesity, common in type 2 diabetes, are characterized by CMD even in the absence of signs and symptoms of cardiac ischaemia or dysfunction. In human obesity, and especially in abdominal obesity, perivascular and epicardial adipose tissue accumulate around the coronary vasculature and heart and become inflamed.[53]

3.2 Hypertension

Hypertension intensifies CMD through functional and structural alterations in the microcirculation. Clinical studies provide evidence that microvascular hallmarks of hypertension are inward remodelling of resistance arteries and microvascular rarefaction , [54] determinants of microvascular resistance reducing myocardial blood flow.

Chronic hypertension, obesity, and diabetes gradually reduce kidney function and are strong risk factors for chronic kidney disease (CKD), with high cardiovascular complications including myocardial hypertrophy, heart failure, and myocardial ischaemia, associated with CMD, as supported by the fact that most patients with CKD do not reach end-stage renal disease, but die from cardiovascular complications.[55]

The molecular connections between diabetes and hypertension are highlighted in Zucker rats, that show how elevated glucose

concentrations activate Rho-kinase, which in turn inhibit internalization and facilitates recycling of angiotensin 1 type (AT1) receptors, leading to increased functional availability of AT1 receptors and sustained angiotensin II-induced arterial constriction.[56]

3.3 Dyslipidaemia

Dyslipidaemia is a major risk factor for microvascular dysfunction. Hypercholesterolaemic patients have clear evidence of reduced CFR , [57] from the early stages of atherosclerosis and before any angiographic evidence of coronary stenosis. Plasma levels of total cholesterol and LDL-C inversely correlate with the FFR and the IMR, independently of the severity of coronary atherosclerosis or the number of diseased vessels. Hypercholesterolaemia has been proven to lead to larger infarcts and adverse cardiac remodelling post-MI by compromising microvascular function in acute STEMI patients.[58]

CMD linked to hypercholesterolaemia primarily relates to impaired endothelium-dependent vasodilation in arterioles. This could be in part due to increased generation of ROS. Interestingly, Ox-LDL dependent changes in large conduit arteries are mirrored at the microvessels, as demonstrated by Hein *et al.*[59] using an experimental model of isolated porcine coronary arterioles. The detrimental effect of OxLDL on endothelium-dependent dilation of arterioles is specifically mediated by the reduction in the expression and function of NO synthase (eNOS) and a low NO-bioavailability, whereas Ox-LDL does not affect the vasodilation of arterioles mediated by components such as cyclooxygenase and cytochromeP-450 monooxygenase nor mediated by endothelial hyperpolarization.[59]

In addition, the pathogenic mechanisms contributing to dyslipidaemia-induced CMD include inflammation, innate, and adaptive immune cell responses, and pro-thrombotic conditions.

4. Sex, microvascular dysfunction, and cardiovascular risk

Women with suspected or confirmed ischaemic heart disease , especially when young, have less atherosclerosis than men and less prevalence of obstructive CAD. However, women at young age have similar prevalence of STEMI than men.[60] Women more often have unexplained higher mortality rates after STEMI, which may be due to smaller vessel size, less collateral flow, more vascular stiffness, and concurrent CMD.[61] In addition, a disproportionate burden of coronary risk factors and comorbidities is a clear feature of women even after adjustment for age. Nevertheless, risk factors have a different impact on mortality depending on age and sex.[37,61] Type 2 diabetes is more likely to be associated with endothelial dysfunction and CMD in women than in men. Similarly, smoking is a well-established cardiovascular risk factor for both sexes, but this is significantly increased in women under age 60, and more markedly in young women who combine the habit of smoking with the use of oral contraceptives.

Indeed, experimental data suggest complex oestrogen-related gender-specific differences in the regulation of NO-mediated microvessel vasomotor function in female and male. These findings may help to better understand the human observations showing significant gender-specific differences in the epidemiology, pathophysiology, clinical features, prognosis, and treatment success between women and men.[62] It seems that sex differences of the coronary circulation are especially pronounced. Therefore, there is an urgent clinical unmet need to develop gender-specific therapeutic and preventive modalities.

In addition, high-risk pregnancies (e.g. gestational hypertension or diabetes) and other factors related to the female reproductive cycle (e.g. polycystic ovary syndrome) give women added vulnerability to develop cardiovascular disease, being recently referred to as sex-specific cardiovascular risk factors.[63] However, their pathological relevance on the microcirculation has not been established.

5. Microvascular endothelial dysfunction and vasospastic angina

Maseri *et al.*[64] suggested 'coronary artery spasm ' as the underlying pathogenic factor of variant angina and Bugiardini *et al.*[65] defined 'vasotonic angina ' as a diffuse epicardial coronary constriction, usually ≥50% of lumen diameter, confined to the distal segments of the coronary arteries and limiting the blood flow supply to the myocardium, which produces myocardial ischaemia in the presence of normal smooth coronary arteries at angiography. In vasotonic angina, the microcirculation is still the major culprit as assessed by coronary blood flow measurements in the coronary sinus, suggesting a concurrent involvement of functional abnormalities in macro- and microvessels of the coronary tree. Thus, endothelial dysfunction is significantly associated with diffuse epicardial vasoconstrictor response following intracoronary infusion of acetylcholine with adverse cardiovascular events.[66] Assessment of endothelial function may identify early changes in vasoactive functions relevant in the development of atherosclerosis rather than identifying atherosclerotic lesions *per se*, as documented in a study in women with chest pain and normal coronary angiograms.[67]

6. Mechanical, cellular, and molecular effectors of microvascular dysfunction

6.1 Haemodynamic forces: pressure and shear stress

The endothelial layer lining the interior of blood vessels is directly exposed to haemodynamic forces. High blood pressure accelerates development of atherosclerotic plaques in large epicardial coronaries and endothelial dysfunction of microvessels of the heart. High intraluminal pressure elicits constrictions of isolated small coronary arteries and arterioles in rats , [68] which serves —in part —to protect the distal microcirculation, including the capillary bed, and oedema development due to high hydraulic/filtration pressure. This, however, imposes higher blood flow velocity and thus shear stress on the endothelium of upstream large vessels, and at branching points.[69]

Haemodynamic forces have a wide spectrum of mechanical and molecular signalling effects on the endothelial cells, impacting their morphology and vasomotor function.[68,70] Thus, the endothelium 'senses ' and 'transduces ' abnormal physical forces into cellular signalling events leading to vascular damage. However, major challenges remain in understanding how interactions between mechanotransduction and chemical signals—such as risk factors—play a pivotal role in eliciting the responses to shear stress in the different vascular beds.

6.2 Inflammation

Systemic inflammation may be a link between CMD and atherosclerosis. Inflammation-mediated endothelial cell activation is characterized by

enhanced production of ROS, and platelet and leucocyte adhesion to the endothelium due to endothelial up-regulation of adhesion molecules (e.g. P-selectin) and loss of endothelial barrier function , [71] with detrimental effects on the epicardial coronary endothelium but also inducing CMD. Sources of ROS/superoxide are NADPH oxidase, xanthine oxidase, mitochondrial respiration, in addition to uncoupled eNOS.[72] ROS/superoxide production plays a key role in the attenuation of NO bioavailability, as described above and it has been linked to the inflammatory response, possibly invoking stress-activated protein kinases such as c-Jun N-terminal kinases, in hypercholesterolaemic mice.[73]

The impact of inflammatory endothelial activation is less clear for other endothelial dilator mechanisms, specifically the endothelium-dependent dilator principle that relies on hyperpolarization (EDH-type dilation). The EDH-type dilation is most important in the microcirculation and therefore must be considered in the setting of CMD. Experimental studies using different animal models of cardiovascular risk reveal a shift of the endothelial-dilator mechanism from NO to EDH in arterioles rather than a global impairment of the response. Similarly, plasticity in endothelial signalling is found in the human microcirculation in disease , [74] suggesting involvement of further mechanisms.

6.3 Platelet activation

Clinical studies support the relevance of platelets at the microvascular level during ischaemia–reperfusion. Thus, platelets contribute to functional and structural coronary MVO occurring after PCI in a large proportion of patients.[75]

Platelets may compromise blood flow at the microvascular level by forming distal microemboli and by adhering to reperfused capillary or venular endothelium or to attached leucocytes, contributing to the release of vasoconstrictor or toxic molecules and a plethora of inflammatory mediators that further enhance the activation of the endothelial monolayer and the recruitment of circulating leucocytes.[76]

Platelet adhesion occurs in intact inflamed microvessels without the need of exposed extracellular matrix material. Oxidative stress, endothelial cell activation, and the accompanying recruitment of rolling and firmly adherent leucocytes are common features for platelet adhesion to the endothelium in the microcirculation. Molecules mediating this interaction are P-selectin and P-selectin glycoprotein ligand-1 or glycoprotein-Ib and von-Willebrand factor.[77]

Adhering to the endothelial cell lining turns platelets into effectors that boost the inflammatory process. They release a large number of proteins from preformed granules, synthetize bioactive molecules (ROS, thromboxane) or shed them from the membrane (e.g. CD40L). This promotes further activation of endothelial cells that express adhesion molecules such as intercellular adhesion molecule 1 fostering leucocyte–endothelial interaction. The interaction platelet CD40L with endothelial CD40-receptor is specifically important in the induction of inflammation-associated microvascular thrombosis, as demonstrated in venules and arterioles of wild type and CD40/CD40L deficient mice.[78]

In addition to the effects elicited through adhesion to the endothelium, activated platelets present HMGB1 (high mobility group box-1) that enhances production of neutrophil extracellular traps (NETs). A recent experimental study of ischaemia–reperfusion in rat demonstrates that NET-mediated microthrombosis significantly contributes to myocardial 'no-reflow'.[79]

6.4 Autonomic dysfunction

Coronary microvascular tone, defined by the ratio between baseline and maximal vessel diameter, is regulated by several mechanisms including myogenic tone, metabolic control exerted by adjacent cells, endothelial function, and circulating factors, alongside autonomic innervation.[80]

Adrenergic innervation provides a mechanism for vessel tone regulation that is particularly important during exercise, or in the presence of endothelial dysfunction, but it has a negligible contribution to resting vascular tone in the healthy coronary circulation , [81] since coronary flow is mainly modulated by mechanisms of non-neural origin in normal conditions. Adrenergic receptors (α- and β-adrenergic receptors) regulate coronary circulation at the level of vascular endothelial and smooth muscle cells. Increased sympathetic activity results in stimulation of the β-adrenergic receptors, with β_2 being the main adrenoceptors in the coronary microcirculation. Conversely, α-adrenergic stimulation produces vasoconstriction, with exception of the α_2 receptors, which are abundantly distributed in the microvasculature and associate with vasodilation when located on endothelial cells.[82]

Chronic dysregulation of autonomic function is characterized by an imbalance between the sympathetic and parasympathetic systems. Autonomic dysfunction involves increased sympathetic activation, with increased vasoconstriction, and/or damage of the autonomic nerve fibres that innervate the heart and blood vessels, including the microvasculature. In general, adrenergic-derived vasoconstriction is relevant in clinical situations in which normal non-neural vasodilator mechanisms are impaired (i.e. dyslipidaemia, diabetes).[83] The role of the parasympathetic innervation in the coronary microcirculation is debatable, even though activation of vascular endothelial M_3 receptors results in increased NO synthesis and subsequent vasodilation.[84]

Impaired autonomic function resulting in vasoconstriction is associated to CMD after AMI and/or coronary reperfusion procedures. In this regards, an early study showed that left ventricular dysfunction (secondary to transient PCI-induced ischaemia) accompanied by increased coronary resistance and diffuse vasoconstriction was abolished by α-blockers, supporting the hypothesis that neural mechanisms elicit microvascular dysfunction.[85]

7. Assessment of the coronary microcirculation

Adequate methods for direct visualization *in vivo* of the coronary microcirculation both in human and animal are presently lacking. Assessment of CMD is performed with different diagnostic modalities, including non-invasive (positron emission tomography, myocardial contrast echocardiography, cardiac computed tomography, and cardiac magnetic resonance) and invasive (coronary angiography, Doppler-flow wire, CFR, and index of microvascular resistance) techniques, each one carrying specific strengths and weaknesses[86,87] (see Supplementary material online, *Table S3* for specifications).

The ESC has proposed an assessment procedure to reach a more precise identification and diagnosis of microvascular angina (*Figure 5*). In fact, preliminary data show that impaired microcirculatory conductance and arteriolar dysregulation may benefit from different treatments.[1]

Similarly, assessment of CMD in animal models basically include *in vivo* non-invasive imaging, invasive catheter based physiological coronary testing and *ex vivo* investigation based on contemporary histology. Haemodynamic indexes, such CFR, IMR, and zero-flow pressure, have

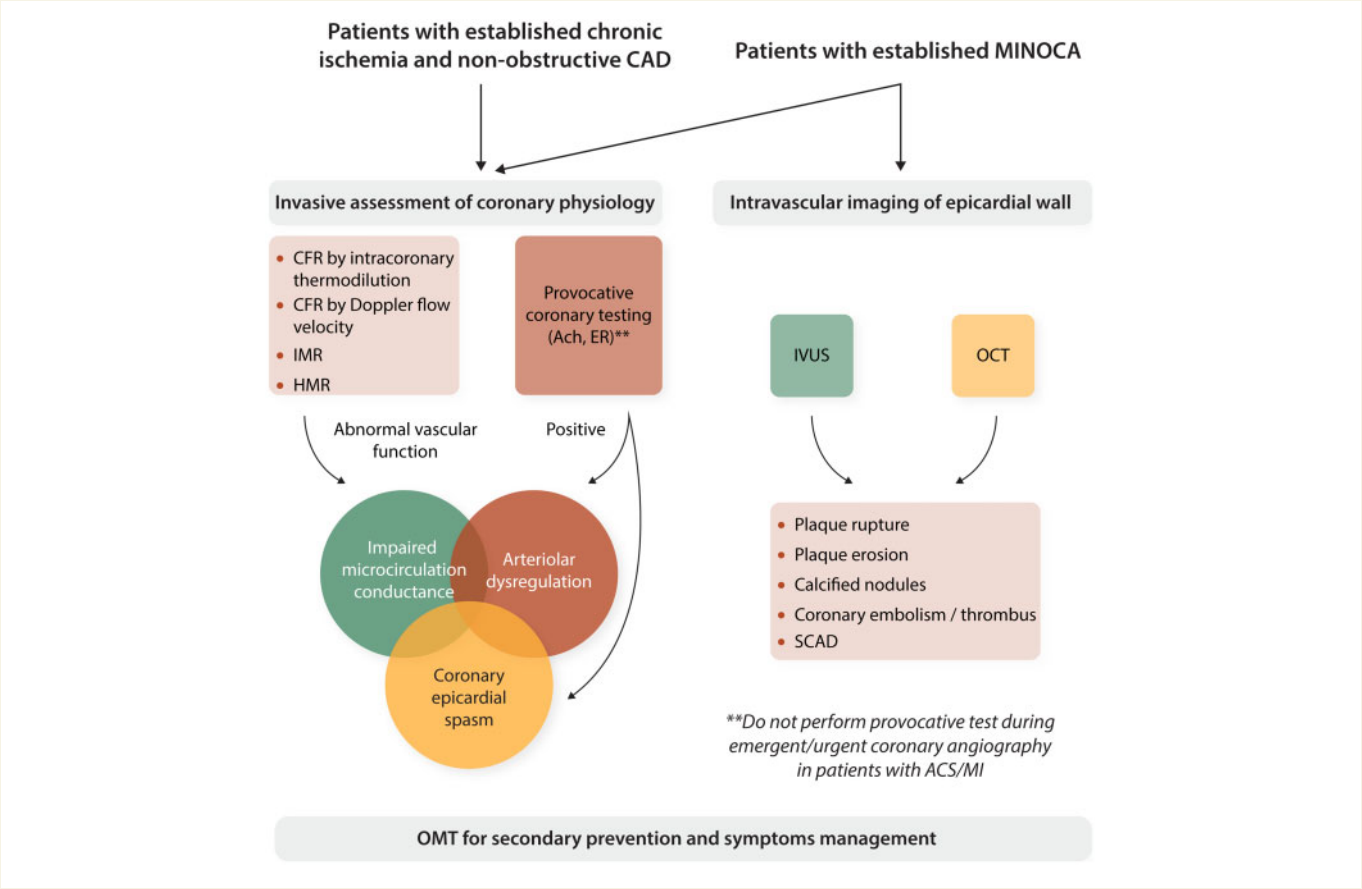

Figure 5 Invasive assessment of coronary physiology and intravascular imaging for patients with non-obstructive CAD. Ach, acetylcholine; ACS, acute coronary syndromes; CAD, coronary artery disease; CFR, coronary flow reserve; ER, ergonovine; HMR, hyperaemic microvascular resistance index; IMR, index of microcirculatory resistance; IVUS, intravascular ultrasounds; MI, myocardial infarction; MINOCA, myocardial infarction with non-obstructed coronary arteries; OCT, optical coherence tomography; OMT, optimal medical therapy; SCAD, spontaneous coronary artery dissection. **Contraindications to provocative coronary testing include emergent coronary angiography in patients with ACS/MI, pregnancy, severe hypertension, severe left ventricular dysfunction, heart failure (New York Heart Association Class III or IV), moderate/severe aortic stenosis, left main coronary stenosis >50%, multivessel CAD, spontaneous spasm during angiography; uncontrolled ventricular arrhythmia, renal insufficiency, severe bronchial asthma; use of isosorbide dinitrate (shortly before).

been proved to be the most reliable to assess coronary microcirculation in swine, a good preclinical model of heart disease.[88]

8. Coronary microvascular ischaemia: the size of the problem

In the last two decades, with the increasing widespread use of imaging techniques for assessing myocardial perfusion, there has been a growing awareness of the fact that many patients suffer myocardial ischaemia and MI , without any atherosclerotic coronary lesion limiting blood flow.

In the literature, the rate of such patients varies widely, since it depends on the definition used for 'non-obstructive CAD '. Some analyses refer to smooth coronary arteries or coronary arteries with minimal lumen irregularities (<20% lumen stenosis),[10,89] others (most studies) to the absence of a significant (≥50%) coronary artery lumen stenosis,[90–93] while others to the absence of any severe (>70%) lumen stenosis.[94,95] Moreover, the estimated prevalence is influenced by gender, clinical syndrome (stable or acute), ethnicity, and other characteristics of the study population, for example veterans with respect to the general population.[94,95]

INOCA: The rate of patients with lumen stenosis <50% in any major coronary arteries, which is the most common definition for INOCA, is approximately 47% in women (from 34% to 65%) and 30% in men (from 14% to 36%).[10,89,96] Supplementary material online, *Table S1* shows data of 10 large cohort studies with angiograms of more than 530 000 patients. One out of two women with chest pain of suspected cardiac origin has non-obstructive CAD compared with one out of three men. However, the concept of INOCA as a woman's disease is an oversimplification, since the absolute number of women and men with non-obstructive CAD is similar.

MINOCA and acute coronary syndromes: Compared with chronic coronary syndrome, a lower but still important percentage of patients with non-obstructive CAD is documented in ACS.[30,90–93,97] Supplementary material online, *Table S2* reports data of 30 international cohorts. The

incidence of non-obstructive CAD, defined by lumen stenosis <50% in any major coronary artery, ranges from 2.2% to 21.8%, being influenced by changes in the definition of AMI over the time (i.e. incorporation of high-sensitivity troponins) and by the clinical context: MINOCA, STEMI, non-STEMI, non ST-elevation ACS, or unstable angina. The incidence is generally lower in STEMI (3%, range 2.2–11.6%) than in NSTEMI (10%, range 8.1–17%) or non ST-elevation ACS (10%, range 9.2–12.1%). MINOCA has a mean rate of 8% (range 2.9–13.8%), with most of the patients NSTEMI. The disease affects both sexes, but women appear more vulnerable and the rate of women with MINOCA (or other ACSs) is from 2 to 3 times higher than that of men.

No-reflow phenomenon: This is a relatively common complication, especially in the setting of STEMI.[41,98–100] The size of the problem depends on its definition. Based on the use of thrombolysis in myocardial infarction (TIMI) grade flow, the occurrence seems to be restricted to less than 5% of the population undergoing primary angioplasty. However, evaluation of myocardial rather than merely epicardial perfusion reveals a much higher prevalence. Thus, despite optimal restoration of TIMI 3 grade blood flow, suboptimal reperfusion is still observed in 20–40% of the STEMI population.[100] Factors associated with impaired reperfusion —despite optimal epicardial reperfusion are advanced age, diabetes, late presentation (>4 h), preprocedural low TIMI flow, and advanced Killip class at presentation.[101–106] Recently, it has been shown that there is a significant sex difference in post-PCI slow flow, being women more affected than men.[37]

8.1 Prognostic relevance of microvascular ischaemia

For a long time, ischaemia with non-obstructive CAD was considered a clinical condition affecting almost exclusively women and not associated with any serious risk of adverse cardiac events. However, several studies published in the early 2000s showed controversial results.[107,108] Al Suwaidi *et al.*[108] reported 4.8% of death, 2.4% of AMI, and 14% of coronary revascularization during 2 years of observation, in 42 stable patients with coronary lumen stenosis <40% and severe endothelial dysfunction. Subsequent analysis of a large cohort of patients with ACS and <50% lumen stenosis, revealing a rate >12% for major cardiovascular events (death, AMI, stroke, revascularization or severe angina) at 1-year , [107] focused attention on the unfavourable prognosis of these patients (women and men) and stimulated further studies in the acute and stable clinical context.

INOCA: The Women's Ischemia Syndrome Evaluation study (WISE) was designed to assess the outcome of women undergoing cardiac catheterization for suspected angina. A major strength of the study was the use of a blinded core-lab for evaluating angiograms by quantitative coronary analysis; a weakness was the enrolment of women only. Sharaf *et al.*[109] observed that cardiovascular death or AMI at 10 years had occurred in 6.7%, 12.8%, and 25.9% of women with no, non-obstructive (>20% but <50% lumen stenosis), and obstructive CAD, respectively. Data from a Danish cohort of 11 223 patients with stable angina also showed an association between non-obstructive CAD and major adverse cardiovascular events. This study found a 1.52-fold and 1.85-fold increased risk of major adverse cardiac events for patients with normal coronary arteries and non-obstructive CAD, respectively, compared to healthy individuals.[10] Furthermore, an increase in risk—regardless of sex—was observed in association with the degree of CAD (normal, non-obstructive and obstructive). Reported mortality rates from 2.4% at 2.5 years , [10] to 5.3% at 6.5 years.[110]

MINOCA: Several studies reported that the outcome of AMI patients with non-obstructive CAD was not favourable as previously thought. Thus, in-hospital death occurs in up to 2% of MINOCA patients , [30,92,111] while at 1-year mortality ranges from 3.1% to 6.4%,[92,112] rising to 10.9% at 5-year.[92] Data from the literature also indicate that during hospital stay, patients with MINOCA often experience left ventricular failure , [93,111,113] while major cardiovascular events (death, AMI, hospital readmission, revascularization, or stroke) occur in 12–14% of these patients at 6–12 months.[90,107]

Vasospastic angina: Perfusion-imaging studies provide evidence that patients with chest pain may actually have reduced CFR in the absence of flow-limiting coronary stenoses. However, impaired CFR does not necessarily imply endothelial vascular dysfunction because the defect can reside in endothelium-independent responses. Abnormalities in coronary microvascular responses to adenosine (which is principally endothelium-independent) do not appear to be predictive of adverse outcomes. Conversely, when impaired CFR is accompanied by coronary endothelial dysfunction, as assessed by acetylcholine testing, it predicts an unfavourable outcome. A number of studies have addressed the long-term prognostic value of endothelial function testing in patients with non-obstructive CAD and demonstrate that endothelial dysfunction is associated with significantly more adverse cardiovascular events.[66,108,114] An investigation of 42 women demonstrated that 30% of those women with chest pain, 'normal ' angiograms, and severe endothelial dysfunction developed CAD during a 10-year follow-up.[67] Another study in 163 patients with 'normal ' coronary angiography and abnormal endothelial function showed an overall event rate of 14% at 48 months. Outcome data included increased rates of cardiovascular death (10% of adverse events), AMI, congestive heart failure, or stroke (21% of adverse events), and angina, revascularization, or other vascular events (69% of adverse events).[114] Loss of endothelium-dependent vasodilation in response to acetylcholine is an early sign of vascular injury ultimately leading to atherosclerosis, and may thus account for the prognostic value of acetylcholine testing.

No-reflow phenomenon: Several studies —using different techniques — have evaluated the prognostic impact of myocardial no-reflow (below TIMI 3 flow), demonstrating an impact on cardiac remodelling and survival.[37,115–119] Among 1548 STEMI patients, impaired reperfusion was associated with a three-fold increase in the relative risk of mortality at 1- year follow-up[99] and similar results were observed at 5 years of follow-up.[116] The prognostic impact of impaired reperfusion on survival and left ventricular remodelling has been demonstrated using myocardial contrast echocardiography (MCE) [117] or cardiac magnetic resonance (CMR).[118]

8.2 Preventive and conventional strategies

To date, no randomized trials comparing therapies for the reduction of adverse cardiac events in patients with angina and 'normal ' coronary arteries have been conducted, and available adverse outcome data are limited to cohort studies. However, the presence of non-obstructive CAD at coronary angiography is increasingly recognized as pathological. Lifestyle changes and risk factor management should be considered essential components of any therapeutic approach for patients with traditional cardiac risk factors, with or without evidence of coronary atherosclerosis. For patients with cardiac chest pain and evidence of ischaemia by perfusion testing, beta-blockers may reduce myocardial oxygen consumption and symptoms. Aggressive therapy with statins and angiotensin-converting enzyme (ACE) inhibitors should be used for patients who qualify for this treatment by the presence of cardiac risk

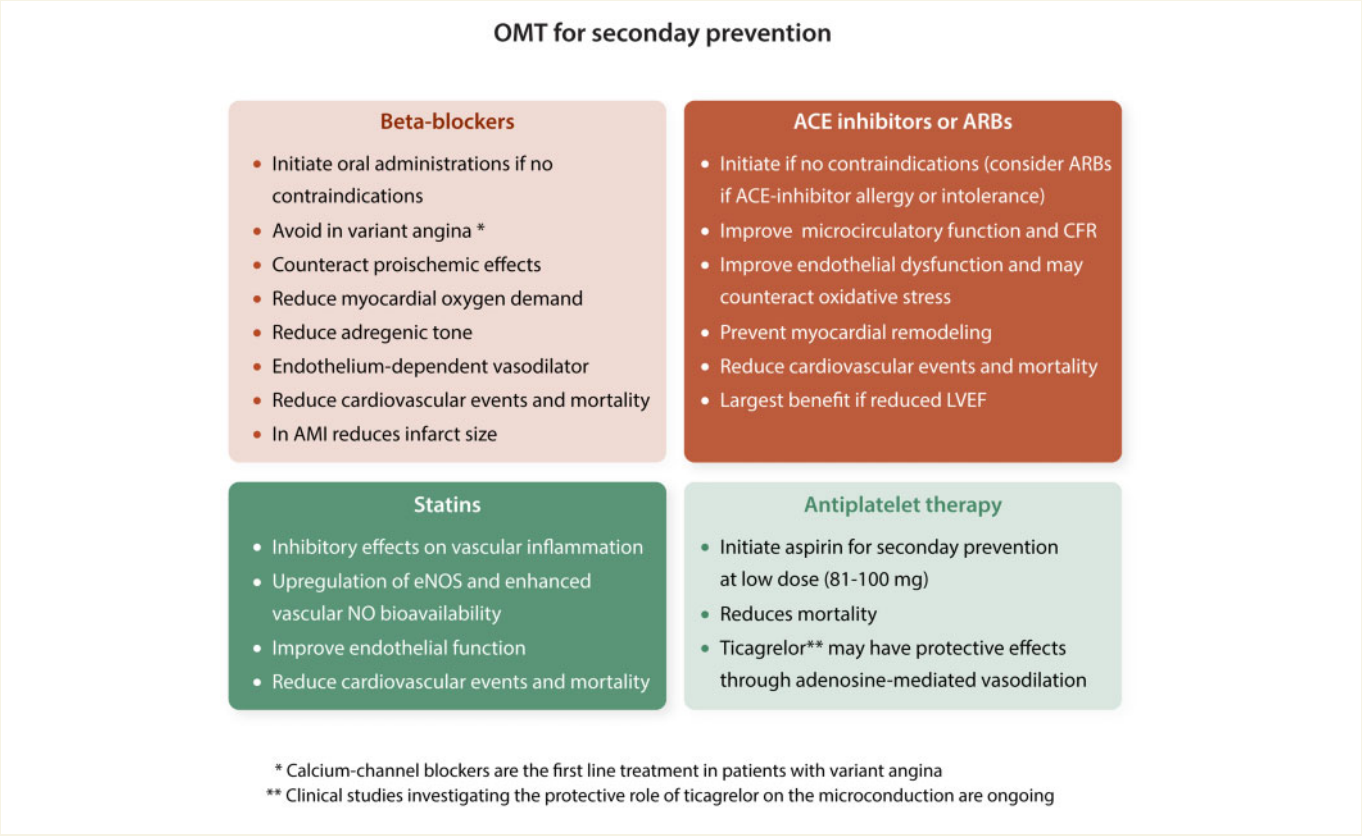

Figure 6 Secondary prevention strategies in patients with microvascular dysfunction or MINOCA. (i) Contraindications to beta-blockers include decompensated heart failure, symptomatic bradycardia and atrioventricular blocks, hypotension, and asthma acute bronchospasm. In case of AMI all the aforementioned with the inclusion of Killip Classes III (acute pulmonary oedema) and IV (cardiogenic shock). (ii) Contraindications to ACE inhibitors or ARBs include acute and worsening renal failure, advanced chronic kidney disease, bilateral renal artery stenosis, hyperkaliaemia, hypotension, history of angio-oedema and hereditary or idiopathic angio-oedema, and pregnancy due to teratogenicity. AMI, acute myocardial infarction; LVEF, left ventricular ejection fraction; MINOCA, myocardial infarction with non-obstructive coronary arteries; OMT, optimal medical therapy. [a]Calcium-channel blockers are the first line treatment in patients with varian angina. [b]Clinical studies investigating the protective role of ticagrelor on the microconduction are ongoing.

factors and have evidence of atherosclerosis or evidence of endothelial dysfunction (*Figure 6*).

Research into novel treatments for CMD is an unmet clinical need. One novel strategy, not yet proven, has been to inhibit Rho-kinase in order to ameliorate CMD and vasospastic angina.[120] In addition, therapeutic targeting of perivascular adipose tissue to stimulate the production of vasoactive, vasorelaxing factors such as adiponectin[121] or hydrogen sulphide[122] could be of benefit.

Platelet inhibitors: Data on the use of platelet inhibitors including aspirin for the treatment of CMD are insufficiently established to provide clinical recommendations. Nonetheless, in patients referred for CAD diagnostic assessment, documentation of CMD often justifies the use of aspirin since typically these patients have also non-obstructive CAD.[123] Ticagrelor, may protect the microcirculation through its adenosine-mediated vasodilator effects.[44,45] Clinical studies investigating its protective role on the microcirculation are ongoing.

ACE inhibitors: Inhibition of the renin-angiotensin axis elicits beneficial vasoprotective effects by improving microcirculatory function and CFR .[124] ACE inhibitors and statin improve endothelial dysfunction, may counteract oxidative stress, and may be of benefit in patients with CMD.[125] Data from EMMACE-2 registry[91] showed that ACE inhibitor therapy was associated with reduced 6-month incidence of mortality in patients presenting with ACS and non-obstructive CAD. This study however, has limitations, including its observational nature, lack of randomization, short duration of follow-up, and inability to assess MI , heart failure, and stroke as outcomes.

Statins: Beyond reducing cholesterol levels, statins have inhibitory effects on vascular inflammation, up-regulate eNOS and enhance vascular NO bioavailability. In patients with normal angiograms and inducible myocardial ischaemia several small randomized trials and case–control studies have shown positive effects of statins on exercise tolerance, exercise-induced reversible perfusion defects , [125–128] endothelial function , [125–127] and quality of life.[125] In the setting of ACS, statin therapy prior to revascularization improves coronary microvascular perfusion in patients with and without STEMI, as assessed by contrast echocardiography in a 30 days of follow-up period.[129] The analysis of eight TIMI trials reported lower rate of death or reinfarction at 30 days in patients with NSTE-ACS and non-obstructive CAD on statin treatment.[130] Moreover, during long-term (>4 years) follow-up, data from SWEDEHEART, which enrolled 9136 patients with MINOCA surviving at least 30 days after the index event, confirmed the beneficial effects of statins on major cardiovascular events (all-cause mortality, MI , ischaemic stroke, and heart failure).[97]

Beta-blockers: Beta-blockers have been shown to be highly effective for reduction of chest pain episodes during daily life. There are several potential mechanisms by which beta-blockers may act in reducing chest pain recurrences as reducing myocardial oxygen demand and inducing endothelium-dependent vasodilation.[131] Exercise training, which increase parasympathetic activity, has shown to be of benefit, indicating the impact of adrenergic modulation.[132] In patients with variant angina beta-blockers should be avoided; whereas calcium-channel blockers are the first line treatment.

Nitrates: Nitrates are effective in inducing vasodilation and relieve angina symptoms, but without consistent findings for patients with non-obstructive CAD.[133]

L-arginine: Long-term, 6-month supplementation of L-arginine, the precursor of NO, improved endothelial function, coronary blood flow, and symptoms in patients with non-obstructive CAD, but not CFR.[134]

9. Concluding remarks

CMD is an intricate part of ischaemic heart disease. It arises from changes in both microvascular function and structure and is most strongly associated with endothelial dysfunction. The dominant underlying functional mechanisms include a loss of NO bioavailability and increased ET-1 vasoconstrictor tone. The structural mechanisms identified to date include inward arteriolar remodelling and vascular rarefaction. Together these increase minimal coronary vascular resistance and reduce maximum myocardial perfusion. As a result, CFR decreases promoting myocardial ischaemia during increases in myocardial oxygen demand. With increasing sophistication to assess coronary physiology in humans, it has become apparent that CMD is highly prevalent and developing therapeutic interventions to reverse functional abnormalities in coronary resistance vessel control could become an important approach to treat ischaemic heart disease.

10. Recommendations and future perspectives

- Both structural and functional defects contribute and interact to cause a progressive impairment of coronary microvascular blood flow. There is a need to clarify the relative impact of each one in the diverse forms of presentation of CMD.
- There are no therapeutic strategies focused on specifically treating CMD and the microvasculature. There is an urgent need to identify novel and specific targets for therapy.
- There is a need to better understand the anatomic and physiologic features that predispose a higher burden of microvascular dysfunction in women.
- There is a need of new research to improve CMD assessment and the diagnostic methodologies now available.
- The management of patients with non-obstructive CAD and ACS or inducible myocardial ischaemia does not currently have a consensus strategy. These patients should be studied for the evaluation of endothelial and CMD , since they may influence outcome.
- Clinical studies and double-blind randomized clinical trials in patients with non-obstructive disease specifically designed to assess the effect of conventional and novel anti-ischaemic therapies are required.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

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