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SPOTLIGHT REVIEW

Pathophysiology and diagnosis of coronary microvascular dysfunction in ST-elevation myocardial infarction

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Abstract

Early mechanical reperfusion of the epicardial coronary artery by primary percutaneous coronary intervention (PCI) is the guideline-recommended treatment for ST-elevation myocardial infarction (STEMI). Successful restoration of epicardial coronary blood flow can be achieved in over 95% of PCI procedures. However, despite angiographically complete epicardial coronary artery patency, in about half of the patients perfusion to the distal coronary microvasculature is not fully restored, which is associated with increased morbidity and mortality. The exact pathophysiological mechanism of post-ischaemic coronary microvascular dysfunction (CMD) is still debated. Therefore, the current review discusses invasive and non-invasive techniques for the diagnosis and quantification of CMD in STEMI in the clinical setting as well as results from experimental *in vitro* and *in vivo* models focusing on ischaemic-, reperfusion-, and inflammatory damage to the coronary microvascular endothelial cells. Finally, we discuss future opportunities to prevent or treat CMD in STEMI patients.

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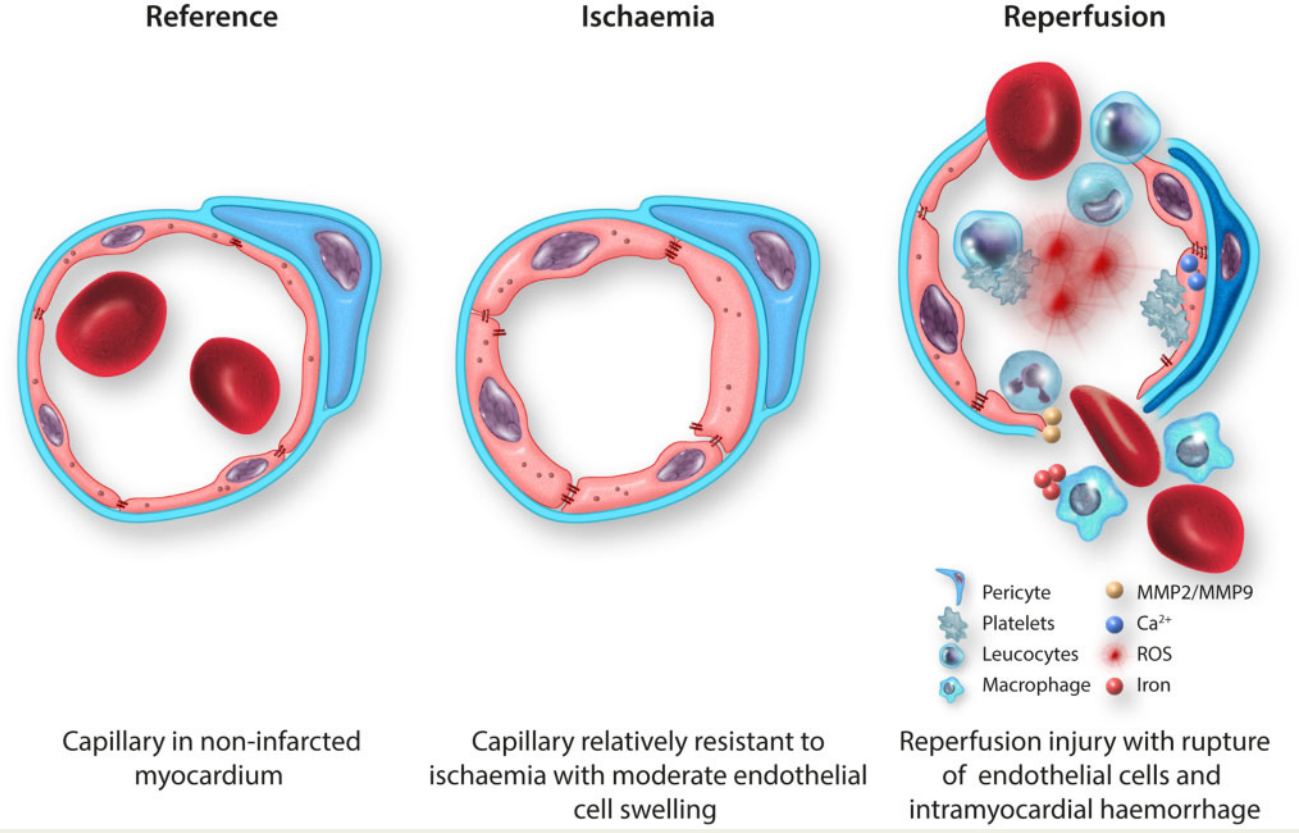
Graphical Abstract



Keywords Coronary microvascular dysfunction • ST-elevation myocardial infarction • Microvascular reperfusion injury • Intramyocardial haemorrhage • Coronary microvascular endothelial cells

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

1. Introduction

The guideline-recommended treatment for ST-elevation myocardial infarction (STEMI) is early mechanical reperfusion of the epicardial coronary artery by primary percutaneous coronary intervention (PCI). In over 95% of the PCI procedures, successful restoration of epicardial coronary blood flow is achieved, which has dramatically reduced mortality rates in STEMI patients.[1] However, despite angiographic evidence of complete epicardial coronary artery patency, in about half of the patients, the perfusion of the distal coronary microvasculature is not fully restored, which is associated with increased morbidity and mortality.[2] In experimental animal models, the extent of poorly or non-perfused regions of the coronary microvasculature evolves during reperfusion.[3,4] This indicates that reperfusion itself paradoxically can have additional harmful effects. The phenomenon in which structural evidence of microvascular damage was linked to poorly or non-perfused regions of the intramural myocardium was first described in 1974.[5] Since then, reperfusion injury has been extensively described in the literature and over the last several years, the coronary microcirculation has evolved from passive bystander to primary target for therapies aiming to diminish reperfusion injury. The exact pathophysiology is still debated,[6] partly due to the fact that—despite multiple efforts—there are still no effective therapeutic options to prevent reperfusion injury in clinical practice.[6–9] In this review, we present an overview of current knowledge on coronary microvascular dysfunction (CMD) in STEMI. We will summarize invasive and non-invasive techniques for the diagnosis and quantification of CMD in the clinical setting, as well as results from experimental *in vitro* and *in vivo* models. Furthermore, we will highlight future opportunities to prevent or treat CMD which could further improve clinical outcome and prognosis of STEMI patients.

1.1 Nomenclature and definitions

Throughout the literature, different terms have been used to describe the phenomenon of diminished myocardial perfusion after reperfused STEMI. As a guide to the reader, we first provide a short overview with nomenclature and definitions (*Table 1*).

1.1.1 No-reflow

Already in 1966, Krug *et al.*[10] observed disturbances of blood supply after removing a ligation of the coronary artery in the cat. However, this

Table 1 Recommendations to use terminology of reperfusion injury

Nomenclature	Definition
No-reflow	*Using coronary angiography:* no, partial or delayed antegrade flow *Using microscopy:* lack of markers for flow, such as carbon black, microspheres or Thioflavin S ***Remark***: the term no-reflow does not provide much information about the pathophysiology
MVO	*Using CMR* with gadolinium-based contrast agents: contrast-enhanced infarct area with contrast-void infarct core ***Remark***: In fact, the contrast-void infarct core reflects severely injured myocardium hampering wash-in of the contrast agent rather than a totally obstructed microvasculature *Using microscopy:* (reversible) distal atherothrombotic embolization, plugging of circulating blood cells, *de novo* microvascular thrombus formation, extravascular compression (e.g. by oedema, IMH)
IMH	*Using CMR* without contrast agents: hypointense infarct core on T2-weighted imaging and/or T2* mapping *Using microscopy:* (irreversible) destruction of capillaries with loss of interendothelial cell junctions, extravasation of erythrocytes into the myocardium
CMD	Umbrella term comprises no-reflow, MVO, IMH, and MVI
MVI	General term for microvascular damage after ischaemia–reperfusion
Reperfusion injury	General term for tissue damage after ischaemia–reperfusion (i.e. not specific for the coronary microvasculature)

CMD, coronary microvascular dysfunction; CMR, cardiac magnetic resonance imaging; IMH, intramyocardial haemorrhage; MVI, microvascular injury; MVO, microvascular obstruction.

observation was not related to microvascular damage in that study. The term no-reflow was first mentioned in a rabbit model of cerebral ischaemia.[11] A few years later, Kloner *et al.*[5] reported coronary no-reflow in a canine model of acute myocardial infarction (AMI). They observed a persistently poor or even absent perfusion in large areas of the reperfused myocardium despite complete epicardial coronary artery patency and linked it to microvascular injury (MVI). Consequently, the term no-reflow has been used to describe the inability to reperfuse regions of previously ischaemic myocardium after re-opening the occluded coronary artery. The following years, this term was maintained mainly based on experimental animal studies using markers for flow, such as carbon black or microspheres, or staining for endothelial cells, such as Thioflavin S. It was assumed that lack of these markers in certain areas of the myocardium represented post-ischaemic no-reflow areas.[12] In patients, coronary no-reflow was reported only years after the first mentioning in experimental studies. Schofer *et al.*[13] provided scintigraphic evidence of no-reflow in a STEMI patient treated with thrombolysis. Shortly after, Bates *et al.*[14] first reported angiographic no-reflow, as estimated coronary blood flow by angiographic contrast density. After the introduction of primary PCI, no-reflow was witnessed much more frequently by immediate angiographic visualization.[15] Because patients with angiographic no-reflow had poor outcomes, absence of this angiographic sign served as a measure of procedural success for many years. However, the term no-reflow does not provide much information about its pathophysiology. In fact, no-reflow refers to multiple manifestations of which microvascular obstruction (MVO), MVI, intramyocardial haemorrhage (IMH), and CMD are the most prominent. Also, in contemporary practice, angiographic no-reflow is only encountered in <5% of patients,[16] which is a clear underestimation when compared to the number of patients with myocardial perfusion deficits on cardiovascular magnetic resonance (CMR) post-STEMI.

1.1.2 Microvascular obstruction

When it became apparent that angiographic no-reflow was not sensitive enough to detect microvascular perfusion deficits, the use of CMR was introduced for this purpose. With CMR, a typical pattern with contrast-enhanced infarct area and contrast-void infarct core was observed and was coined MVO, since it was thought that this was the underlying mechanism preventing contrast to reach the infarct core. It was hypothesized that distal atherothrombotic embolization, plugging of circulating blood cells, *de novo* microvascular thrombus formation and extravascular compression attribute to MVO. However, none of the clinical trials targeting the aforementioned factors have led to positive results,[6,17] indicating that true MVO might only play a limited role in reperfusion injury. Moreover, CMR-defined MVO is reversible in some patients.[18] Furthermore, it has become clear that CMR-defined MVO often reflects MVI comprising complete microvascular destruction and IMH.[19,20] Therefore, the term MVO should be reserved to describe the histologically proven obstruction of microvessels rather than the complete clinical entity of failed primary reperfusion.

1.1.3 Intramyocardial haemorrhage

IMH is an irreversible pathological consequence of severe MVI.[21] Whilst MVO might resolve,[18] e.g. recovery of perfusion with resorption of oedema, IMH represents capillary destruction which is irreversible. Experimentally, reperfusion causes IMH[22,23] and is reflected by the loss of interendothelial cell junctions and extravasation of erythrocytes in the perivascular space.[23] Furthermore, a large overlap was found in size and location of CMR-defined MVO and histologically proven IMH.[19]

1.1.4 Coronary microvascular dysfunction

The pathophysiology of reperfusion injury is of multifactorial origin and may include impaired vasomotor function, MVO, MVI, IMH, and inflammation.[6] Therefore, the term CMD in STEMI better reflects the multifaceted pathophysiology of myocardial reperfusion deficits caused by a constellation of pathological mechanisms. We note that the term CMD is currently also used in the setting of ischaemia and no obstructive coronary artery disease. In the present review, we will use the term CMD (in STEMI) unless specific knowledge on the pathophysiological substrate is available.

1.2 Incidence and prognosis of CMD in STEMI patients

Occurrence of CMD after reperfused STEMI is associated with unfavourable clinical outcome and prognosis. As stated above, surrogates of CMD in STEMI can be measured with angiography or CMR. Using angiography, CMD is often denoted no-reflow. Angiographic no-reflow was reported in only 2.7% of STEMI patients. Patients with no-reflow showed

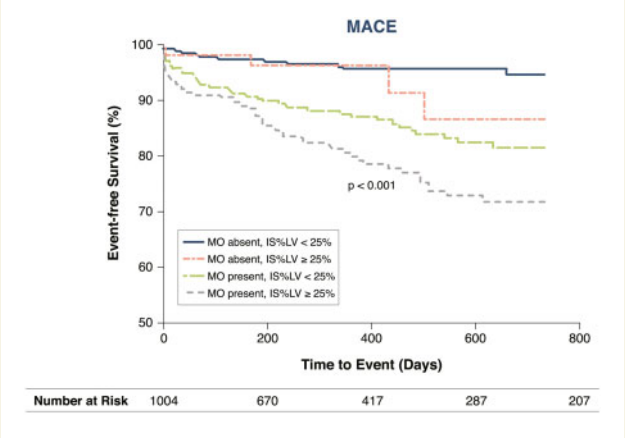


Figure 1 Relationship between CMR-defined microvascular obstruction, infarct size, and major adverse cardiac events. IS, infarct size; IS%LV, infarct size as percentage of left ventricle; LV, left ventricle; MACE, major adverse cardiac events; MO, CMR-defined microvascular obstruction. Values are Kaplan–Meier estimates in patients with IS%LV ≥25% vs. <25%, grouped by the presence or absence of MO, indicating the time to MACE during 2 years of follow-up. MACE is defined as a composite of cardiac death, myocardial reinfarction, and new congestive heart failure at 2 years of follow-up. Reprinted with permission from Van Kranenburg *et al.*[2]

higher in-hospital mortality and higher rates of reinfarction, cardiogenic shock, and heart failure compared to patients without no-reflow.[16] Using CMR, which is nowadays the most used entity to assess myocardial damage, CMD is denoted MVO or IMH. MVO, which was defined by a contrast-devoid infarct core, was present in 54.9% of patients with angiographic optimal flow [defined as thrombolysis in myocardial infarction (TIMI) 3 flow]. The presence of MVO was found to be an independent predictor of major adverse cardiac events (MACE) at 2 years of follow-up. Furthermore, the presence of MVO has even incremental detrimental effects in addition to the extent of infarct size at 2 years of follow-up (*Figure 1*). In addition, MVO was an independent predictor of cardiac death.[2] Not only the presence of MVO but also the extent of MVO is associated with poor clinical outcome. Both presence and extent of MVO assessed 15 min after contrast injection were identified as the strongest independent predictors of MACE, defined as a composite of death, myocardial reinfarction and congestive heart failure, and the individual outcome mortality.[24] Furthermore, IMH, which was defined by a contrast-devoid infarct core on T2-weigthed imaging, was present in 34% of patients. IMH was an independent predictor of MACE.[25]

2. Invasive and non-invasive techniques for diagnosis and quantification of CMD in STEMI

Different methods—both invasive and non-invasive—have been described to diagnose and/or quantify CMD in STEMI. We will summarize these methods and discuss their main strengths and limitations.

2.1 Invasive approaches

2.1.1 Angiographic methods

2.1.1.1 TIMI flow. TIMI flow is an angiographic visual scoring system grading epicardial antegrade flow. No antegrade flow is denoted TIMI 0 flow. Complete antegrade flow, including the distal bed, is denoted TIMI 3 flow. TIMI 1 and 2 flow are respectively denoted antegrade flow beyond the obstruction but without distal perfusion, and complete antegrade, but delayed flow.[26] Post-procedural TIMI 3 flow is associated with fewer MACE and better survival compared to TIMI ≤2 flow.[27,28] Although the angiographic TIMI flow assessment is fast and easy to perform, more than half of the patients with normal TIMI 3 flow show CMR-defined MVO.[29] Thus, preserved TIMI flow does not necessarily guarantee adequate microvascular perfusion and is not sensitive enough to assess post-ischaemic CMD. Therefore, in current practice with fast mechanical reperfusion by primary PCI in the majority of patients and hence TIMI 3 flow, the predictive power of post-procedural TIMI flow is less evident. Because of a high interobserver variability and poor interobserver agreement in grading TIMI flow, the corrected TIMI frame count (CTFC) has been introduced, providing a quantitative index to assess coronary flow by counting the number of frames required for contrast to reach a standardized distal landmark. Advantages of CTFC are its high reproducibility, low intra-, and interobserver errors and it enables a quantitative estimation of flow.[30–32] However, the prognostic value of the CTFC is not clear.[33–36]

2.1.1.2 Myocardial blush grade. Myocardial blush grade (MBG) is an angiographic visual scoring system, assessing myocardial perfusion by myocardial contrast blush after injection, grading from no myocardial contrast density to normal myocardial contrast density in the infarct-related coronary artery.[37] MBG has been defined as 0, in case of no myocardial blush or contrast density or persisting myocardial blush; 1, with minimal myocardial blush or contrast density; 2, with moderate myocardial blush or contrast density, but less than that obtained during angiography of a contralateral or ipsilateral non-infarct-related coronary artery; and 3, with normal myocardial blush or contrast density, comparable with that obtained during angiography of a contralateral or ipsilateral non-infarct-related coronary artery. MBG provides additional diagnostic value to TIMI flow; of patients presenting with post-procedural TIMI 3 flow, two-third of patients had MBG 0–1. Furthermore, it has prognostic value; multivariate logistic regression showed that MBG is associated with long-term survival rates independent of TIMI flow.[37] CMR-defined MVO was less frequently observed in patients with MBG ≥2 compared to MBG 0–1.[38] However, similar to preserved TIMI flow, preserved MBG does not necessarily indicate adequate microvascular perfusion. In patients presenting with MBG 2–3, still, a substantial proportion of patients showed CMR-defined MVO.[29,38]

2.1.1.3 Computer-assisted myocardial blush quantification by quantitative blush evaluator. Vogelzang *et al.*[39] described a novel assessment of myocardial perfusion through computer-assisted myocardial blush quantification by quantitative blush evaluator (QuBE). Although QuBE was an independent predictor of mortality in the work by Vogelzang *et al.*,[39,40] these results have yet to be investigated by other groups. Furthermore, ~20% of angiograms could not be assessed using QuBE due to overlapping vessels or panning movements.

2.1.1.4 Fluoroscopy assisted scoring of myocardial hypoperfusion. A novel non-invasive estimate for coronary blood flow is the fluoroscopy assisted scoring of myocardial hypoperfusion (FLASH) represented by FLASH flow and FLASH ratio. FLASH flow is calculated by contrast passage velocity multiplied with the mean cross-sectional area. FLASH ratio is calculated as the relative difference between FLASH flows in the infarct-related coronary artery compared to that in a non-infarct-related coronary artery. In STEMI patients, FLASH flow is significantly lower in the

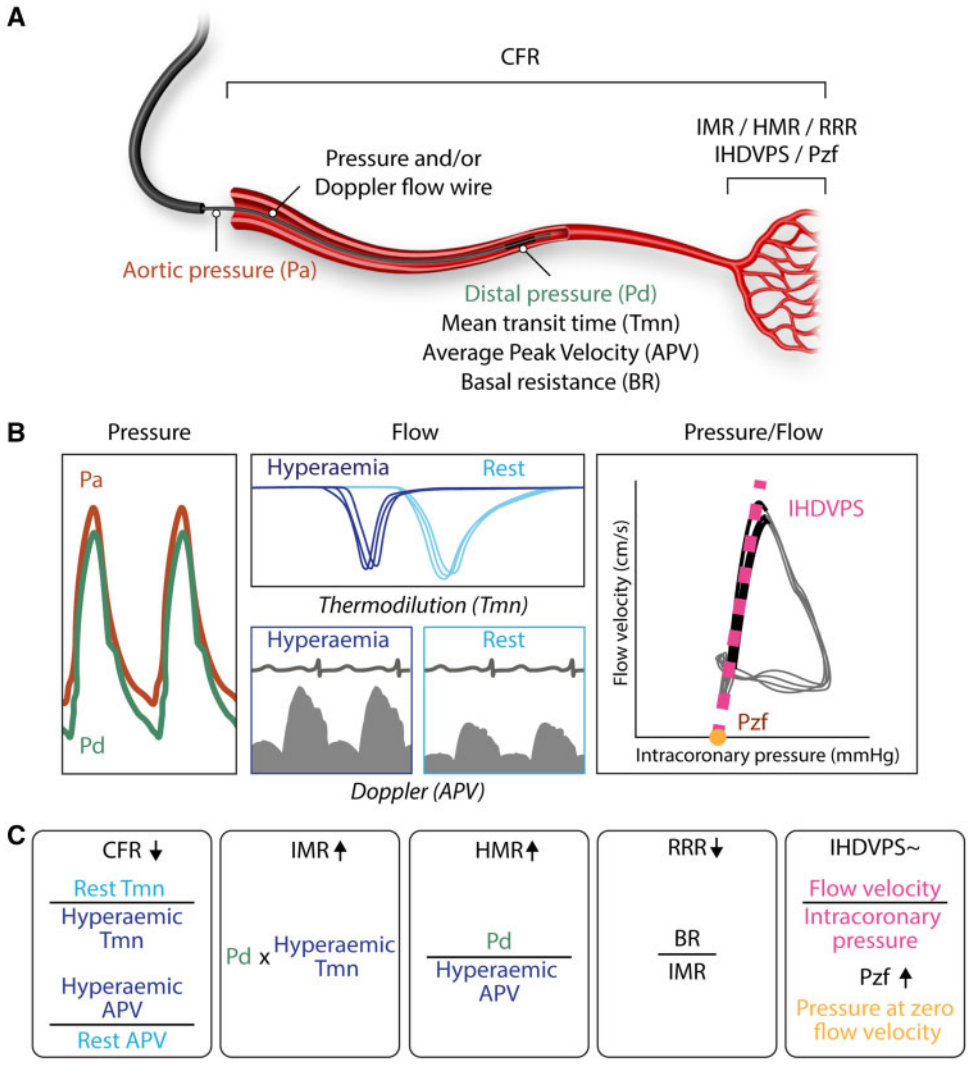


Figure 2 Intracoronary physiology: invasive measurements for coronary microvascular function after ST-elevation myocardial infarction. (*A*) Schematic overview of Pa, Pd, Tmn, APV, BR, CFR, IMR, HMR, RRR, IHDVPS, and Pzf. (*B*) With an intracoronary pressure and/or Doppler flow wire the following indices can be derived: Pa (red), Pd (green), BR, hyperaemic (dark blue lines) and rest (light blue lines) Tmn, hyperaemic (dark blue box) and rest (light blue box) Doppler APV, IHDVPS (pink dotted line) and Pzf (yellow dot). The right box shows the pressure–flow velocity loops and the calculation of the linear relationship between pressure and flow velocity over the mid-late phases of diastole (coloured in black). The higher the values of IHDVPS (the β term in the equation $y = a + \beta x$, where y = flow velocity and x = intracoronary pressure), the better the conductance of the microcirculation. Pzf is calculated from the intercept of the regression line with the pressure axis. (*C*) Formulas for CFR, IMR, HMR, RRR, IHDVPS, and Pzf. The arrow indicates higher or lower values for that formula when coronary microvascular dysfunction is present. Evidence on the ability of IHDVPS to reflect coronary microvascular dysfunction is controversial. See text for details. APV, average peak velocity; BR, basal resistance, CFR, coronary flow reserve; HMR, hyperaemic microvascular resistance; IHDVPS, instantaneous hyperaemic diastolic flow velocity–pressure slope; IMR, index of microcirculatory resistance; Pa, aortic pressure; Pd, distal pressure; Pzf, pressure at zero flow velocity; RRR, resistive reserve ratio; Tmn, mean transit time.

infarct-related coronary artery as compared to non-infarct-related coronary arteries. FLASH ratio is an independent predictor of cardiac mortality (optimal cut-off value -49% for cardiac mortality at 6 months) and might be more sensitive than TIMI flow and MBG.[41] However, FLASH has not been validated in an independent prospective cohort.

2.1.2 Intracoronary physiology

With intracoronary pressure and/or flow wires the function of the coronary microcirculation can directly be assessed. First, intracoronary nitroglycerin is infused to ensure complete epicardial vasodilation. Subsequently, the function and the capacity of the distal microvascular bed are assessed by infusion of vasodilators like adenosine that act specifically on the microcirculation. Measurements to assess the functionality of the coronary microcirculation include coronary flow reserve (CFR), index of microcirculatory resistance (IMR), hyperaemic microvascular resistance (HMR), resistive reserve ratio (RRR), instantaneous hyperaemic diastolic flow velocity–pressure slope (IHDVPS), and coronary zero-flow pressure (Pzf) (*Figure 2*).

2.1.2.1 Coronary flow reserve. CFR provides information on the epicardial and microvascular compartments and can be derived using

intracoronary wires with Doppler or thermodilution techniques.[42] Using Doppler, CFR is defined as the ratio of hyperaemic blood flow divided by resting blood flow. Using thermodilution, CFR is defined as the ratio of mean transit time (Tmn) in rest divided by Tmn during hyperaemia. As CFR reflects both the epicardial- and the microvasculature, CFR can be used to assess the microcirculation in the absence of an epicardial stenosis. A value of <2.0 has a sensitivity of 79% and specificity of 34% for CMR-defined MVO.[43] CFR corresponds with the extent of CMR-defined MVO.[44] CFR measured in the infarct-related coronary artery is a prognostic marker for left ventricular function recovery after AMI and is associated with long-term mortality.[45,46] A limitation of CFR is its reproducibility. Because resting flow is included in the calculation, changes in heart rate, blood pressure, and left ventricular contractility influence the CFR value.[47]

2.1.2.2 Index of microcirculatory resistance. While CFR reflects the combined epicardial and microvascular resistance, IMR and HMR specifically assess microvascular resistance. The distal coronary pressure multiplied with the hyperaemic Tmn provides IMR. The hyperaemic Tmn is the average of three separate transit time measurements after intracoronary injection of room temperature saline during adenosine-induced hyperaemia. Calculation of IMR is not affected by variations in hemodynamic conditions (because resting measurements are not required) or the severity of epicardial stenosis.[48] A meta-analysis found that IMR is significantly increased in patients with CMR-defined MVO.[49] IMR is more closely associated with MVO and clinical outcomes than TIMI myocardial perfusion grade or CFR.[50] With regard to clinical outcome, a higher IMR is associated with worse outcome like adverse left ventricular remodelling at 6 months[50] and even death.[51] A limitation of IMR is the manual injection of saline, which can be a source of variability.

2.1.2.3 Hyperaemic microvascular resistance. HMR is calculated as the distal pressure divided by the mean Doppler flow velocity at peak hyperaemia simultaneously measured using a coronary guidewire with a combined pressure sensor and Doppler transducer.[52] An HMR value >2.5 mmHg cm^{-1} s has been shown to be indicative of CMR-defined MVO with a sensitivity of 71% and a specificity of 63%. Furthermore, an HMR value >2.82 mmHg cm^{-1} s is a strong predictor of a composite of death and rehospitalization for heart failure.[53] The role of HMR in STEMI has been studied less compared with IMR, and a limitation of HMR is the complexity of acquiring high-quality measurements with the Doppler wire.

2.1.2.4 Resistive reserve ratio. RRR is a measure of the ability to achieve maximal coronary hyperaemia. RRR is the ratio of basal resistance to IMR. Whereas IMR is a measure at peak hyperaemia and reflects structure, RRR quantifies the vasodilator response of the coronary microcirculation to a hyperaemic stimulus such as adenosine.[54] In a prospective study of 50 patients with stable angina, 40 patients with STEMI and 50 patients with non-STEMI (NSTEMI), no difference was observed in RRR between non-culprit vessels in stable angina and either culprit vessels in stable angina or NSTEMI. However, RRR was significantly lower in STEMI patients.[55]

A novel measurement to assess microvascular function is thermodilution-based absolute flow and resistance measurement using continuous infusion of saline through a dedicated coronary catheter. This potentially reduces introduced variability by avoiding the manual injection of saline. Although larger studies in STEMI are awaited, early studies have shown the feasibility of this approach in both stable angina and STEMI patients and recently the technique was validated with positron emission tomography.[56,57]

2.1.2.5 IHDVPS and coronary Pzf. Alternative approaches to interrogate the coronary microcirculation are the measurement of the IHDVPS and Pzf. In this approach, coronary pressure–flow velocity loops are generated. As shown in *Figure 2B*, a linear relationship between pressure and flow values exists during the mid-late phases of diastole. The slope of the regression line obtained over this period expresses microcirculatory conductance. The described index, called IHDVPS, correlates well with structural changes in the coronary microcirculation.[58] However, evidence on its ability to reflect reperfusion injury in patients with STEMI is controversial, with some studies suggesting a potential role[59] and others failing to demonstrate its value as a predictor of CMD in the context of STEMI.[52,60] A second index derived from the mid-late diastole linear relationship between pressure and flow values is Pzf. This index, which stems from the concept of vascular waterfalls,[61] is calculated by extrapolation from the regression line relating pressure and flow values that have been described above for IHDVPS calculation. The value of Pzf in assessing reperfusion injury associated to STEMI is that, from a theoretical standpoint, it should reflect of the intraluminal pressure required to maintain patent compressible elements against extravascular compression. In the context of STEMI, extravascular compression may result from increased interstitial pressure secondary to myocardial haemorrhage and/or oedema. Pzf may also reflect extravascular compression due to raised intraventricular filling pressures resulting from acute myocardial injury.[62] Available evidence is largely consistent on the predictive value of Pzf in the acute phase of STEMI in predicting subsequent myocardial injury and infarct size.[52,60,63,64]

2.2 Non-invasive approaches

2.2.1 CMR imaging

CMR plays a pivotal role in the characterization of post-infarction myocardial injury, most essentially with the use of a contrast agent. The CMR gadolinium-based contrast agent is a contrast medium with extracellular distribution, meaning that after injection it slowly diffuses in areas with increased extracellular or interstitial space, such as the acutely infarcted myocardium with ruptured cell membranes. While the contrast agent washes out from the viable myocardium with intact myocytes, the washout in infarcted myocardium is delayed, causing a hyperenhanced bright signal on the T1-weighted images, hence the name delayed contrast enhancement (LGE).[65] In some patients, the hyperenhanced infarcted myocardium surrounds a subendocardial area of decreased signal in the core of the infarct, which slowly becomes hyperenhanced over time if repeated images are acquired.[29] These patients with so-called MVO on CMR have worse functional recovery after STEMI, with the best predictive value if the images are taken 10–15 min after contrast injection.[66] This predictive value is better than angiographic assessment or the electrocardiogram.[29] Since the amount of MVO is dependent on timing of the image acquisition due to the pharmacokinetic dynamics of the contrast agent, it is clear that the area with MVO as visualized with CMR is not a well-defined area with a totally obstructed microvasculature, but reflecting severely injured myocardium hampering wash-in of the contrast agent. In a recently published recommendation paper for CMR endpoints in experimental and clinical trials, LGE has been defined as the most important primary endpoint, with MVO and left ventricular ejection fraction (LVEF) as main secondary endpoints since these were strongly associated with MACE and had consistent evidence in multiple studies.[67]

With the use of additional newer techniques, CMR provides further infarct characterization, with a surrounding oedematous border zone, an

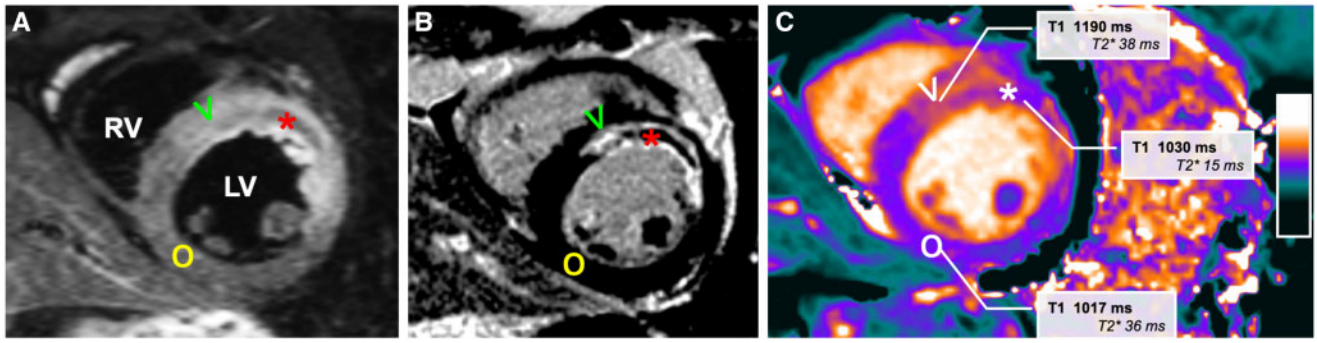


Figure 3 CMR images of a patient after acute anterior myocardial infarction. Typical example of a patient after acute anterior myocardial infarction with microvascular injury, demonstrating hyperintense oedematous myocardium on the T2-weighted image (*A*, V) compared to remote non-infarcted myocardium (O), with the corresponding delayed contrast-enhanced image showing the infarcted hyperenhanced infarcted myocardium (*B*, V) with a hypointense infarct core (asterisk) with microvascular injury. The area with microvascular injury has low T1 on the non-contrast T1 map (*C*, asterisk) and low T2*, whereas the area of infarction has increased T1 relaxation times and normal T2* decay times, not containing haemoglobin breakdown products. LV, left ventricle; RV, right ventricle.

infarcted central zone with ruptured myocytes and in some patients a necrotic core with MVI and haemorrhage.[68] The most used non-contrast technique is T2-weighted imaging, in which the oedematous myocardium causes prolongation of T2 decay times, and therefore, a relatively higher signal in acutely infarcted myocardium compared to remote myocardium. Additionally, this technique has been used for the assessment of IMH as well,[69] since the paramagnetic effects of haemoglobin cause an attenuated low signal which correspond to regions of haemorrhaged infarcts on pathology.[70] Newer mapping techniques allow a quantitative per pixel analysis of the myocardium without contrast, quantifying the degree of oedema (T2 mapping), myocyte loss (T1 mapping), and if present haemorrhage (T2* mapping). T1 relaxation and T2 decay times are prolonged in acutely infarcted tissue, whereas T1 and T2 are lower in the infarct core if IMH is present. T2* is merely reduced in the presence of IMH, and is not altered in remote or infarcted myocardium, and currently accepted as the preferred method to identify haemorrhage in the infarct core[71] (*Figure 3*). For future trials investigating treatment strategies to prevent reperfusion injury, it is, therefore, important to include T2* mapping and T1/T2 mapping to study its effect on the different infarct components.[67]

3. Experimental models of CMD in STEMI

Experimental models show that microvascular endothelial cell dysfunction plays a prominent role in reperfusion injury. In the following paragraphs, subsequently ischaemic damage, reperfusion damage, vascular permeability, IMH, inflammatory damage, and the role of platelets and pericytes will be reviewed (*Figure 4*).

3.1 Ischaemic damage to endothelial cells

Endothelial cells are relatively resistant to hypoxia. *In vitro* studies with human umbilical vein endothelial cells (HUVEC) showed that three-quarter of all cells survived 24 h of hypoxia[72] and more than half of all cells survived 48 h of hypoxia,[73] indicating that endothelial cells tolerate prolonged periods of hypoxia before destructive damage occurs. Besides some reduction in endothelial cell viability, hypoxia can have several reversible effects on endothelial cell function, including decreased release of vasoactive substances such as the vasodilator endothelial nitric oxide synthase (eNOS) and increased release of vasoconstrictors such as endothelin-1 (ET-1), which could hamper flow restoration.[74] However, after permanent left anterior descending artery (LAD) occlusion, *ex vivo* acetylcholine-induced coronary vasodilation was preserved,[75–77] suggesting sufficient coronary vasodilator reserve after ischaemia. With regard to endothelial cell injury, hypoxia up to 6 h resulted in reduced levels of malondialdehyde, which is a marker for oxidative stress, and increased levels of the antioxidant superoxide dismutase,[72] suggesting even an initial protective effect of hypoxia on endothelial cells. Prolonged periods of hypoxia *in vitro* resulted in some destabilization of tight junctions[78] and adherence junctions,[79] but 90 min of LAD occlusion did not reduce the number of interendothelial cell junctions,[23] indicating that longer hypoxic periods are necessary to induce vascular leakage. Ultrastructurally, the coronary endothelial cell layer remained intact after ischaemia,[76,77] even after 6 h of ischaemia.[76] Addition of *in vivo* reperfusion, however, resulted in prominent and immediate coronary endothelial cell injury with subendothelial bleb formation and disruption of interendothelial cell junctions.[23,77] With regard to the coronary microvasculature, permanent occlusion of the circumflex branch showed mild endothelial cell swelling, some loss of pinocytotic vessels and caveolae, and nuclear chromatin condensation, but no completely occluded capillaries.[80] Also, in a rat model of 30 min of ischaemia without *in vivo* reperfusion, no clear damage to the coronary microvascular endothelium was observed, whereas 30 min of ischaemia in combination with 60 min of *in vivo* reperfusion led to visible damage to the coronary microvasculature with prominent extravasation of erythrocytes in the myocardium.[23]

3.2 Reperfusion damage to endothelial cells

A 90 min proximal coronary artery occlusion followed by reperfusion was associated with perfusion defects detected by injecting the

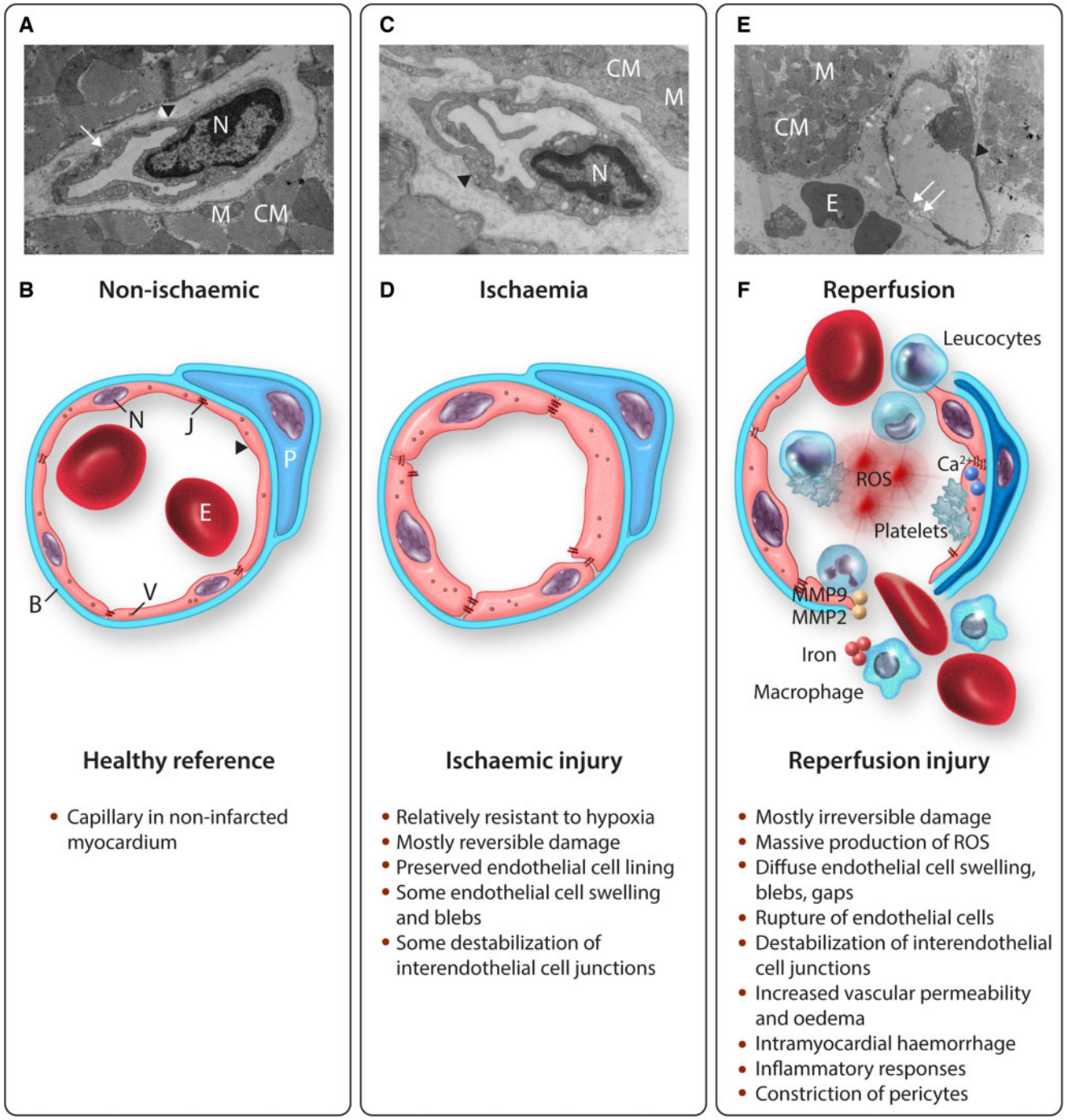


Figure 4 Coronary microvascular endothelial cell dysfunction in reperfused STEMI. (*A*) TEM image of a capillary in non-infarcted myocardium (healthy reference) of a rat[23] with thin endothelium, preserved interendothelial cell junctions and numerous pinocytotic vesicles (small arrow), surrounded by a basement membrane; 17 500×. (*B*) Schematic overview of a healthy reference capillary with an attached pericyte. (*C*) TEM image of a capillary in permanently ischaemic myocardium of a rat[23] with a preserved endothelial cell lining and some localized endothelial cell swelling, surrounded by a basement membrane; 24 500×. (*D*) Schematic overview of a capillary in permanently ischaemic myocardium with some diffuse endothelial cell swelling and some destabilization of interendothelial cell junctions. (*E*) TEM image of a completely ruptured capillary—beyond the phase of endothelial cell swelling with blebs—in reperfused myocardium of a rat[23] with ruptured endothelial cell lining (small arrows) and intramyocardial haemorrhage; 5800×. (*F*) Schematic overview of completely ruptured capillary in reperfused myocardium with massive production of ROS, elevated levels of cytosolic calcium activating the contractile elements of endothelial cells, platelet adhesion and aggregation, destabilization of interendothelial cell junctions, intramyocardial haemorrhage, and numerous inflammatory cells. ▶, endothelial cell; B, basement membrane; CM, cardiomyocyte; E, erythrocyte; J, interendothelial cell junctions; M, mitochondrion; MMP, matrix metalloprotease; N, nucleus; P, pericyte; ROS, reactive oxygen species; V, pinocytotic vesicle.

fluorescent dye, Thioflavin S, into the vasculature after a clamp was removed from the epicardial coronary artery. These perfusion defects were observed within seconds of initial reperfusion and were most prominent in the subendocardial layer of the left ventricular wall. A detailed ultrastructural analysis was performed to try to determine the cause of these microvascular perfusion defects. The most consistent finding was the presence of membrane bound 'blebs' that appeared to pinch off of the endothelial lining of capillaries and small vessels, and obstruct the lumen. These 'blebs' really represented localized areas of endothelial swelling. Other evidence of endothelial damage was present including more diffuse endothelial swelling, loss of pinocytotic vesicles within the endothelium, visible breaks of the endothelial lining with platelet, fibrin tactoids, and neutrophils in the same regions, extracellular erythrocytes, and rouleaux formation. Occasional obstructions to capillaries were present that appeared to be due to compression from adjacent swollen cardiac myocytes and may have also contributed to no-reflow.[5] Subsequent studies showed that the ultrastructural abnormalities to the microvasculature appeared to occur after irreversible injury to the myocytes had already occurred.[81] In addition, the no-reflow zones appeared to occur within areas of the heart that were already dead as assessed by triphenyl tetrazolium chloride (TTC) staining. However, the size of the no-reflow zone expands during the first few hours after epicardial coronary artery patency is established,[3] suggesting that no-reflow is a true manifestation of reperfusion injury.

Thus, reperfusion is paramount to CMD in STEMI and prolonged periods of ischaemia can enhance the extent of reperfusion injury.[82–85] Part of this reperfusion injury might be explained by the massive production of reactive oxygen species (ROS), in turn, leading to oxidative stress and additional vascular damage. In the vasculature, the main sources for ROS are xanthine oxidase, nicotinamide adenine dinucleotide phosphate oxidase, uncoupled eNOS and vascular adhesion protein-1.[86] These ROS sources have been frequently proposed as targets for cardioprotection, but the effects of antioxidants on reperfusion injury are conflicting and results of experimental animal studies failed to be translated into the clinical setting. However, most of these studies focused on reperfusion injury to cardiomyocyte function rather than endothelial cells.[87–89]

3.3 Vascular permeability and oedema

Reperfusion is associated with myocardial oedema.[8,90] Oedema already begins during ischaemia and occurs intracellularly (due to a loss of function of energy requiring ion pumps) as well as in the interstitium (due to an increase in osmolality as a result of metabolite accumulation).[8,90] Upon reperfusion, the coronary reactive hyperaemia with normo-osmotic blood causes a further worsening of oedema, but within hours interstitial oedema begins to wane as washout of catabolites eliminates the osmotic gradient between the interstitium and the intravascular compartment.[91] The early wave of oedema is subsequently followed by a second wave of oedema that appears to be principally the result of an increase in coronary microvascular permeability, possibly as part of influx of inflammatory cells and healing response of the infarct zone.[92] Indeed, serial CMR imaging studies have demonstrated such bimodal pattern of myocardial oedema after reperfusion both in pigs[82,93,94] and in humans,[95,96] especially when IMH was present.[18] The results of these studies showing highly variable levels of oedema early after reperfusion also raise concern regarding the validity of studies attempting to estimate the area at risk, using T2-weighted CMR imaging techniques.[90] Myocardial oedema is not only a consequence of a severe ischaemia–reperfusion insult but, in turn, can contribute to the perturbations in coronary microvascular perfusion during reperfusion by increasing the extravascular compressive forces acting on the coronary microcirculation.[90,97] Studies in swine suggest that this may be particularly prominent during the early wave of oedema, at a time (2 h of reperfusion) when IMH was not yet apparent.[82] In contrast, at 24 h of reperfusion, haemorrhage was now very prominent and was a more likely contributor to the MVO at this time point, when oedema had dissipated.[82]

The endothelial disruption and the subsequent extravasation of cells upon reperfusion are likely facilitated by destabilization of the cellular junctions.[8,90] Following reperfusion, the endothelium undergoes significant alterations in calcium-homeostasis, characterized by elevated levels of cytosolic calcium activating the contractile elements of endothelial cells.[98] The resultant contraction promotes the formation of intercellular gaps, resulting in enhanced permeability to large molecules.[98] In addition, cytokine release further impairs the stability of cell junctions and subsequently increases vascular permeability, via activation of Src and dissociation of the VEGFR2/VE-cadherin complex.[99] Mice deficient for the vascular permeability modulator angiopoietin-like 4 (ANGPTL4) showed increased vascular leakage assessed by Evans blue dye and fluorescent microspheres, disruption of adherence junctions assessed by VE-cadherin staining, increased phosphorylation of Src kinase, and dissociation of the VEGFR2/VE-cadherin complex. Furthermore, these mice showed increase in myocardial infarct size, oedema, haemorrhage, and inflammation. Conversely, injection of recombinant ANGPTL4 reduced vascular permeability, infarct size, no-reflow, and haemorrhage.[100] Interestingly, a recent study in mice undergoing ischaemia–reperfusion reported that losartan inhibited the phosphorylation of Src and VE-cadherin resulting in increased VEGFR2-Src-VE-cadherin complex formation, increased cell surface VE-cadherin, and inhibition of vascular hyperpermeability. These effects were accompanied by a decrease in myocardial infarct size as well as IMH, oedema, and inflammation.[101] Besides destabilization of cellular junctions, reperfusion results in increased matrix metalloproteases (MMP)-2 and -9, which are able to degrade collagen in basement membranes, also promoting vascular permeability.[102,103]

3.4 Intramyocardial haemorrhage

The most severe form of MVI is IMH, which is a result of destruction of coronary capillaries and subsequent extravasation of erythrocytes into the myocardium.[21,23] In a porcine model of ischaemia–reperfusion, histological analysis showed an infarct core with disruption of the microvasculature, extravasation of erythrocytes, necrosis, and cellular debris. The border zone contained besides necrosis and cellular debris, infiltration of leucocytes, granulation tissue, but more importantly a preserved microvasculature.[19] Also, in primates, IMH was present in the core zone and decreased towards the border zone.[104] CMR-defined IMH was first described in dogs with 4 h of coronary artery occlusion followed by 1 h of *in vivo* reperfusion. IMH, which was described by a hypointense core of a hyperintense area on T2-weighted imaging, was present in 80% of animals. Furthermore, IMH assessed by CMR was closely correlated with IMH assessed by histology.[105] In addition, combined porcine and patient data show overlap in MVO and IMH, assessed by histology, LGE-imaging, and T2-weighted imaging on CMR.[19] Pathophysiologically, IMH will result in destruction of haemoglobin with subsequent iron release in the myocardium. In a canine ischaemia–reperfusion model, histological assessment in the acute phase of AMI showed extravasation of erythrocytes with iron deposition in the infarct core zone, but not in the border zone. In addition, 2 months after AMI, histological assessment showed persistent iron deposition in the infarct core zone. Furthermore, newly recruited macrophages were colocalized with the iron depositions in the

core zone, suggesting an ongoing inflammatory response.[106] In the study of Carrick et al.[107] all patients with IMH assessed by T2* imaging had CMR-defined MVO. Bulluck et al.[108] showed that more than 80% of patients with CMR-defined MVO had IMH. IMH can occur already within 4–12 h after primary PCI[107] and show a peak incidence in the first week in both patient and experimental animals.[18,82,107,108]

3.5 Inflammatory damage to endothelial cells

In one experimental study with a large non-atherosclerotic epicardial coronary artery, several changes were observed when a segment of that artery was deprived of blood flow by mechanical clamping both proximally and distally for 3 h followed by reperfusion.[109] Light microscopic analysis of the reperfused segment revealed an influx of neutrophils in the intimal and medial layer of the epicardial coronary artery. Neutrophil influx was not observed in the control non-ischaemic coronary bed. Ultrastructural analysis revealed that neutrophils in the reperfused coronary arteries often appeared to be located between the endothelial cells of the luminal surface of the vessel and the elastic lamina. Neutrophils are also primary sources for MMP-9, in turn, promoting vascular leakage.[103,110]

Also, in other animal models of coronary occlusion, reperfusion was shown to promote rapid adhesion of leucocytes to the microvascular endothelial lining.[111] Aggregates of these immune cells, with platelets, or in the form of neutrophil extracellular traps (NETs) can directly impede blood flow, and cause further endothelial damage by the release of pro-inflammatory cytokines.[112,113] In addition to this histological evidence of leucocyte adhesion early in reperfusion, several observational studies in patients with AMI reported an association between the presence and extent of CMR-defined MVO and circulating inflammatory markers.[6] In more detail, the extent of MVO is associated with the inflammatory circulating neutrophil-to-lymphocyte ratio on admission,[114] as well as peak concentration of leucocytes[115] and with high levels of classical monocytes.[116] More recently, a transcriptome analysis of whole blood in STEMI patients revealed that the extent of late MVO was associated with a differential higher expression of inflammatory genes.[117] In addition, the presence of MVO is associated with levels of inflammatory cytokines, including high-sensitive C-reactive protein,[115] interleukin-8,[118] and interleukin-6.[119] In murine[112,120] and porcine[121,122] models of coronary occlusion, several anti-inflammatory interventions have proven effective in limiting MVO and/or reducing final infarct size, including depletion of neutrophil DNase to reduce NET formation, and inhibition of transcription factor nuclear factor kappa B (NF-κB). In contrast, however, all clinical trials that addressed these inflammatory components in patients with AMI did not show any benefit, which was recently reviewed by Rymer and Newby.[123] For example, inhibitors of the CD11/CD18 integrin receptor in STEMI patients did not result in smaller infarct size, nor improved microvascular flow.[124] In addition, studies targeting the complement system did not show beneficial effects.[123] It is hypothesized that effective anti-inflammatory therapies in animal models failed to be translated in human, probably partly because of the method of coronary artery occlusion (coronary artery ligation vs. coronary artery occlusion by the process of atherothrombosis) and also partly because of the timing of anti-inflammatory drug administration. Some significant beneficial effects in animal models were observed when drugs were administered before the onset of ischaemia. However, in humans, this strategy is not possible in the setting of STEMI.[123] Moreover, most inflammatory processes appear to be secondary in the cascade of reperfusion injury, such as macrophage recruitment to iron depositions and the aggregation of leucocytes and platelets.

3.6 Platelets

The role of platelets in reperfusion injury was recently reviewed by Ziegler et al.[125] Several platelet-associated mechanisms of reperfusion injury have been proposed, including aggregation of platelets and formation of microthrombi in microvessels, release of vasoconstrictors like thromboxane A2, aggregation of platelets and leucocytes leading to additional pro-inflammatory leucocyte infiltration, release of extracellular vesicles (i.e. exosomes, microvesicles, and apoptotic vesicles) which can enhance inflammation, and stimulation of ischaemia-sensitive spinal afferent nerves. Aspirin and P2Y12 inhibitors have shown some cardioprotective effects but mainly because of their effects beyond platelet inhibition, such as their anti-inflammatory effects. The cardioprotective effects of glycoprotein IIb/IIIa (gpIIb/IIIa) inhibitors are unclear, but their use was accompanied by bleeding complications. Presently, the Platelet Inhibition to Target Reperfusion Injury trial (PITRI; NCT03102723) investigates whether the addition of the P2Y12 inhibitor cangrelor on top of conventional dual antiplatelet therapy is more effective in reducing infarct size and CMR-defined MVO than conventional dual antiplatelet therapy alone.

3.7 Pericytes

Pericytes are perivascular cells that are present along the endothelium lining the capillaries and venules.[126] These types of cells are difficult to distinguish from other mural cells and as a consequence have been defined by a combination of morphology, anatomical localization, co-expression of markers, and functional characteristics.[126,127] Pericytes are particularly recognized for their role in the diseased cerebral microvasculature, where they have been proposed to impede reperfusion by constricting capillaries upon ischaemia.[128] Pericytes are also abundantly present in the heart[126] and a role for pericytes in myocardial reperfusion injury was studied by O'Farrel and Attwell.[128] LAD occlusion for 45 min followed by 15 min of reperfusion showed that 40% of the capillaries in the reperfused area were not perfused, which was accompanied by a 50% reduction in microvascular perfusion, despite complete epicardial coronary artery patency. Staining for leucocytes and erythrocytes failed to provide evidence for their presence at blocked capillaries. In contrast, unperfused capillaries were found in proximity of pericytes, identified as neuron-glial antigen 2 (NG2) positive cells. Furthermore, in close proximity of the soma of these pericytes, the capillary lumen diameter was reduced,[128] leading to the hypothesis that cardiac pericytes actively constrict upon ischaemia, resulting in reduction of microvascular perfusion. Although the underlying molecular and cellular mechanisms by which pericytes contract are still incompletely understood,[129] it is of interest that intravenous adenosine infusion started just prior to reperfusion attenuated constriction of capillaries at the site of pericytes, thereby reducing capillary blockage from 40% to 30%,[123] while significantly improving microvascular perfusion.[121,128]

3.8 Reperfusion injury to endothelial cells in other organs

MVI is not limited to the heart but has also been described in the brain, kidney, and intestines. Although information on effects of ischaemia without reperfusion to endothelial cells is scarce, reperfusion itself has additional harmful effects on the cerebral, renal, and intestinal microvasculature and these effects are comparable to the coronary

microvasculature. In the majority of studies however, the results are based on qualitative data rather than quantitative data.

Rats with a permanent middle cerebral artery occlusion showed in particular in the first 2 h of ischaemia endothelial nucleus swelling, moderate endothelial cell swelling, and an increase in endothelial mitochondria. After 12 h of ischaemia, endothelial cell necrosis became clearly apparent,[130] indicating that ischaemia alone has limited effects on the cerebral endothelial cells. Similarities and differences in proposed mechanisms of cerebral and coronary reperfusion injury are recently reviewed by Kloner *et al.*[131] After reperfusion, both organs show endothelial cell swelling causing blebs, inflammatory responses, and increased vascular permeability.

Mice subjected to renal ischaemia showed mild changes in the actin cytoskeleton of endothelial cells compared to controls. During ischaemia, interendothelial cell junctions were preserved. Reperfusion, however, resulted in disruption of the interendothelial cell junctions and subsequently increase in vascular permeability.[132] Also rats subjected to renal ischaemia showed preserved vascular permeability.[132] Renal reperfusion injury was reflected by endothelial- and interstitial cell swelling.[133]

Rats subjected to one hour of superior mesenteric ischaemia without reperfusion showed a decrease in NOS activity. Reperfusion was unable to restore this decreased NOS activity.[134] In rats with 15 min of iliac artery ligation, intraluminal protrusions, but no endothelial gaps were described. Reperfusion resulted in severe endothelial damage and presence of leucocytes.[135]

4. Association between traditional cardiovascular risk factors and CMD in STEMI

Many patients presenting with AMI have one or more traditional cardiovascular risk factors such as diabetes mellitus, hypertension, hypercholesterolaemia, and smoking. These risk factors have also been associated with endothelial dysfunction, mainly explained by a decreased bioavailability of NO and an increased generation of ROS, as reviewed by Brunner *et al.*[136] Potentially, pre-existing endothelial dysfunction increases the risk of reperfusion injury to the microcirculation (*Table 2*).

4.1 Pre-existent diabetes and/or hyperglycaemia

Diabetes mellitus is a metabolic disease with macro- and microvascular complications. Patients with diabetes have a 2–4 times higher risk of cardiovascular complications and mortality.[150] Systemic endothelial dysfunction[136] and even CMD[151,152] seem to play a key role in these cardiovascular complications. As endothelial dysfunction plays a prominent role in the cardiovascular complications of diabetes mellitus, an association between pre-existing diabetes, hyperglycaemia, and post-ischaemic CMD seems plausible. Insulin at physiological concentration increases coronary microvascular perfusion.[153] The presence of absolute or relative insulin resistance in diabetes mellitus may, therefore, contribute to worsening microvascular function. However, in STEMI patients with pre-existing diabetes, diabetes *per se* is not associated with CMD.[137–141] In contrast to pre-existing diabetes, blood glucose level and hyperglycaemia on admission, which is not uncommon during AMI, are both associated with CMR-defined MVO.[137,138,140] A prospective trial in non-diabetic patients with first presentation of STEMI confirms that hyperglycaemia on admission was associated with more pronounced CMR-defined MVO.[142] This suggests that hyperglycaemia, rather than diabetes mellitus, plays a pivotal role in CMD. Acute hyperglycaemia impairs endothelium-dependent vasodilatation in the brachial artery in healthy humans.[154] In a hyperglycaemic canine model, endothelium-dependent coronary microvascular dilatation was impaired, whereas endothelium-independent coronary microvascular dilatation remained unaffected.[155] Hyperglycaemia exerts its actions on endothelial cell function by perturbations in cell signalling, enhanced toxic metabolites, altered osmolarity, in turn, leading to oxidative stress and inflammatory cytokines.[156] Therefore, it was hypothesized that lowering blood glucose level prior to or directly after primary PCI results in less CMD. In diabetic and non-diabetic rodent ischaemia–reperfusion models, treatment with metformin starting at the onset of reperfusion resulted in a reduction of myocardial infarct size.[157–161] However, in a porcine ischaemia–reperfusion model, combined intravenous and intracoronary metformin started at the onset of reperfusion failed to reduce infarct size.[162] Combined results of two large randomized controlled trials OASIS-6 and CREATECLA show that glucose–insulin–potassium therapy did not improve survival after AMI and was even harmful in the first days post-AMI. However, the extent of CMD was not assessed.[163] Whereas treatment with exenatide, a glucagon-like peptide-1 analogue, resulted in smaller infarct size in animal models,[164–166] it failed to limit infarct size in STEMI patients.[167–169]

4.2 Pre-existent hypertension

Hypertension is strongly related to cardiovascular mortality.[170] Also, STEMI patients with pre-existing hypertension have worse prognosis in terms of MACE and mortality.[143,144] Essential hypertension is associated with impaired endothelium-dependent vasodilatation,[171–174] mainly by reduced bioavailability of NO and increased production of ROS. However, assessment of reperfusion injury with invasive intracoronary parameters showed no association between pre-existing hypertension and TIMI flow, CFR, IMR, and RRR.[144] Using LGE and T2-weighted imaging on CMR, there was no association between pre-existing hypertension and MVO[143,144] or IMH.[144]

4.3 Hypercholesterolaemia

Hypercholesterolaemia is associated with reduced bioavailability of NO.[174] Rabbits undergoing ischaemia–reperfusion after a cholesterol-enriched diet for 3 days showed increased infarct size as determined by TTC staining and increased MVO zone as determined by Thioflavin S.[175] In patients, however, the relation between pre-existing hypercholesterolaemia and the development of post-ischaemic CMD is less clear. Reindl *et al.*[146] showed that hypercholesterolaemia was associated with CMR-defined MVO, although the odds ratio was only 1.02. Iwakura *et al.*[145] showed that the incidence of no-reflow determined by contrast echocardiography was comparable between patients with and without hypercholesterolaemia. Effect of chronic pre-treatment with statins on CMD is conflicting.[145,146] However, early high-dose statin treatment with rosuvastatin or atorvastatin did not reduce myocardial injury in STEMI patients.[176,177]

4.4 Smoking

Smoking has a negative effect on vascular function, including microvascular function.[178] Besides endothelial dysfunction,[179] smoking produces a more thrombogenic state.[180] How active smoking influences outcome with regard to post-ischaemic CMD is less clear and even conflicting.[147–149] In a multicentre, prospective study with active smokers presenting

Table 2 Clinical studies investigating the association between pre-existing cardiovascular risk factors and the presence and extent of coronary microvascular dysfunction after acute myocardial infarction

	Study	Variables	Measure of CMD	Main results
Diabetes	*Iwakura* (2003)[137] (*n* = 146)	Patient interview or medical record, abnormal oral glucose tolerance test, or HbA1c ≥6.5%	MCE	Categorized to MVO: no difference in diabetes and HbA1c
	Eitel (2012)[138] (*n* = 411)	Known diabetes	LGE-CMR	Categorized to diabetes: no difference in presence and extent of MVO
	Zia (2014)[139] (*n* = 52)	Known diabetes or new diagnosis with HbA1c ≥6.5% or fasting BG ≥7.0 mmol/L	MVO by T1; IMH by T2* maps	Categorized to diabetes: no difference in presence and extent of MVO or IMH
	Ota (2015)[140] (*n* = 93)	Known diabetes or abnormal oral glucose tolerance test	LGE-CMR	Categorized to MVO: no difference in diabetes
	Reinstadler (2017)[141] (*n* = 792)	Known diabetes	LGE-CMR	Categorized to diabetes: no difference in presence and extent of MVO
Blood glucose level (BG)	*Iwakura* (2003)[137] (*n* = 146)	BG on admission; hyperglycaemia [BG ≥160 mg/dL (∼8.9 mmol/L)]	MCE	Categorized to MVO: BG was higher in MVO; BG and hyperglycaemia are independent predictors of MVO (OR 1.02 and RR 12.1)
	Eitel (2012)[138] (*n* = 411)	BG on admission	LGE-CMR	Graded relationship between BG on admission and MVO
	Ota (2015)[140] (*n* = 93)	BG on admission	LGE-CMR	BG is an independent predictor of MVO (OR 1.014)
	Jensen (2011)[142] (*n* = 107)	Hyperglycaemia (BG ≥7.8 mmol/L on admission). Diabetic patients excluded	LGE-CMR	Hyperglycaemia is an independent predictor of the presence and extent of MVO
Hypertension	*Reinstadler* (2016)[143] (*n* = 795)	Antihypertensive treatment or ≥3 SBP values >140 mmHg on at least two different days	LGE-CMR	Categorized to hypertension: no difference in presence and extent of MVO
	Carrick (2018)[144] (*n* = 324)	Antihypertensive treatment or ≥3 SBP values >140 mmHg on at least two different days	LGE-CMR, ST- resolution; IMH by T2* maps, intracoronary measurements	Pre-existing hypertension was dubious associated with IMH (OR 1.81 with CI 0.98–3.34). Blood pressure on admission was not associated with MVO
Hypercholesteraemia	*Iwakura* (2006)[145] (*n* = 293)	Previously diagnosed or total cholesterol >220 mg/dL (∼5.7 mmol/L)	MCE	Categorized to hypercholesterolaemia: no difference in incidence of no-reflow Statin pre-treatment was an independent predictor of no-reflow (OR 0.22)
	Reindl (2017)[146] (*n* = 235)	Blood samples on admission	LGE-CMR	Categorized to MVI: total cholesterol and LDL were higher in MVI Hypercholesterolaemia was associated with MVI (OR 1.02 with CI 1.01–1.02) No association with statin pre-treatment and MVI
Smoking	*Symons* (2016)[147] (*n* = 471)	Regularly smoking the past 12 months	LGE-CMR; IMH by T2	Categorized to smoking: smokers had higher incidence of IMH (31% vs 20%) but comparable MVO; smoking was associated with IMH (OR 2.13–2.17)
	Reinstadler (2017)[148] (*n* = 727)	Patient interview	LGE-CMR; IMH is not described	Categorized to smoking: no difference in presence and extent of MVO or IMH
	Haig (2018)[149] (*n* = 196)	≥100 cigarettes in life and currently routinely smoking	LGE-CMR; IMH by T2* maps	Categorized to smoking: no difference in presence and extent of MVO or IMH Smoking was associated with IMH when corrected for IS (OR 2.76)

BG, blood glucose; CI, confidence interval; IMH, intramyocardial haemorrhage; IS, infarct size; LGE-CMR, late gadolinium-enhanced cardiac magnetic resonance imaging; LV, left ventricle; MVI, microvascular injury; MVO, microvascular obstruction; *n*, number; OR, odds ratio; RR, relative risk ratio, SBP, systolic blood pressure.

with their first STEMI, the incidence of IMH on T2-weighted imaging on CMR was higher compared to non-smoking STEMI patients, whereas MVO was comparable between groups. In addition, smoking was independently associated with IMH when corrected for infarct size and MVO.[147] In contrast, Reinstadler *et al.* and Haig *et al.*[148,149] showed that presence and extent of MVO and IMH was comparable between smokers and non-smokers. However, the latter study did show that smoking was associated with IMH when corrected for infarct size.[149]

5. Treatment of CMD in STEMI in clinical practice: past, present, and future possibilities

In the practice guidelines of the European Society of Cardiology,[181] a Class I recommendation is consistent with clear evidence and general agreement. Class IIa implies some incomplete or conflicting evidence, and the balance favours 'should be considered'. A Class IIb recommendation indicates the efficacy is less well-established consistent with a 'may be considered' recommendation.

5.1 Established clinical evidence

CMR-defined MVO is related to the size of infarction. From a pragmatic perspective, clinical measures to limit the severity of AMI, notably timely presentation to hospital to limit the duration of ischaemia, will intuitively be helpful to limit infarct size and prevent CMD on a case by case basis.[182]

In a randomized, controlled trial of intra-coronary vasodilator therapy in patients with acute coronary syndromes, Vijayalakshmi *et al.*[183] compared intracoronary administration of heparinized saline, intracoronary verapamil (0.5 mg in 10 mL of heparinized saline) or intracoronary adenosine (30 µg in 10 mL of heparinized saline). They observed similar improvements in coronary blood flow as reflected by the TIMI Frame Count (TFC) acutely and left ventricular wall motion at 1 and 30 days after PCI in the verapamil and adenosine groups. However, a systematic review by Su *et al.*[184] concluded there is insufficient evidence.

More recently, in the REFLOW-STEMI trial[185] compared with standard primary PCI, high-dose intracoronary adenosine (2–3 mg), and intracoronary sodium nitroprusside (0.5 mg in total) during primary PCI did not reduce CMR-defined infarct size or MVO. In a per-protocol analysis, high-dose intracoronary adenosine was associated with an increase in infarct size, a reduction in LVEF and an increase in MACE at 6 months. In patients with no- or slow reflow, these findings point to a 'do not treat' recommendation for higher doses of intracoronary adenosine.

The practice guidelines indicate that gpIIb/IIIa inhibitor therapy should be considered for bailout therapy for patients with slow- or no-reflow.[181] This is a Class IIa recommendation supported by a Level C recommendation since there are no clinical trials. Options for gpIIb/IIIa inhibitor therapy include abciximab, tirofiban, and epitifibitide. These drugs are given by either intravenous or intracoronary routes, initially with a bolus followed by a maintenance infusion for 12–24 h.

5.2 Evidence that does not support clinical use

There are four other trials of intracoronary reduced dose lytic therapy, including T-TIME (NCT02257294), STRIVE (NCT03335839), and the smaller OPTIMAL trial (NCT02894138) using alteplase and RESTORE-MI (ACTRN12618000778280) using tenecteplase. The recently published T-TIME study is a Phase 2 clinical trial of low-dose adjunctive intracoronary fibrinolysis with alteplase in reperfused STEMI.[17] By targeting thrombus within the infarct-related artery and microcirculation with fibrinolytic therapy the aim was to restore microvascular blood flow at the earliest point after coronary reperfusion. CMR-defined MVO, as reflected by the amount (percentage of left ventricular mass) and percentage of affected patients, was not reduced by alteplase.[17] The results do not support the use of low-dose alteplase either routinely in patients at risk of CMD or in those patients with slow- or no-reflow. The potential for intracoronary lytic therapy to prevent or treat CMD remains to be determined.

Routine deferral of stent implantation is also not associated with favourable results[186] and is not recommended in practice guidelines.[181]

5.3 Future possibilities

In the DEFER-STEMI pilot trial,[187] STEMI patients with successful reperfusion by initial thrombectomy and/or balloon angioplasty were randomized to deferred stenting with an intention-to-stent 4–16 h later or conventional treatment with immediate stenting. Fewer patients in the deferred stenting group had no/slow-reflow or intra-procedural thrombotic events. TIMI coronary flow grades at the end of PCI and myocardial salvage index at 6 months were higher in the deferred stenting group. CMR-defined MVO was numerically reduced, but the difference was not statistically significant. The paradigm of a selective approach to deferred stenting is being prospectively assessed in the PRIMACY study (NCT01542385). The investigators derived the Bayesian prior probability of efficacy with patient-level data from the DEFER-STEMI (UK),[187] MIMI (France),[188] DANAMI-3 (Denmark),[187] and INNOVATION (South Korea)[189] trials, which all tested the delayed stenting hypothesis. The investigators aim to determine whether a selective strategy of deferred stenting increases the probability of early urgent target vessel revascularization in the short-term and whether or not there may be meaningful protective effects against heart failure in the longer-term.

In smaller scale studies, intracoronary nitrate and intracoronary nicorandil[190] have been associated with favourable improvements in TFC. These treatments are routinely used by clinicians to treat patients with no-reflow. However, larger trials will be needed to substantiate practice guideline recommendations.

The Early-Beta blocker Administration before reperfusion primary PCI in patients with ST-elevation Myocardial Infarction (EARLY-BAMI) trial involved a randomized, double-blind, matched-placebo-controlled clinical trial of intravenous metoprolol (intravenous bolus of metoprolol, 5 mg) in STEMI patients.[191] There were no between-group differences in infarct size at 30 days post-MI or MACE. The effect on CMD was not reported.[191] The Effect of Metoprolol in Cardioprotection During an Acute Myocardial Infarction (METOCARD-CNIC) trial randomized patients with anterior STEMI who were undergoing primary PCI within 6 h of symptom onset to receive intravenous metoprolol (15 mg) or not (control). The first dose of oral metoprolol was scheduled within 24 h of admission. Infarct size was reduced and LVEF at 6 months was increased compared to control treatment.[192] The incidence of MACE at 2 years was comparable between the early metoprolol and control group.[193] Metoprolol treatment was associated with a 40% reduction in the extent of CMR-defined MVO and the mechanism might involve inhibition of neutrophil activation.[194] These trials present seemingly conflicting results. The differences in the results might be explained by patient selection, design and power. Overall, the results support the case for a substantive, randomized, placebo-controlled, double-blind, clinical trial of intravenous metoprolol before primary PCI.

Ischaemic conditioning, which is defined by either repeated brief episodes of mechanical ischaemia/reperfusion or by pharmacological

prevention of the opening of the mitochondrial permeability transition pore before primary PCI or at the onset of reperfusion, has been championed as a novel cardioprotective therapy.[195,196] However, recent trials of intravenous cyclosporine,[197] TRO40303,[198] and post-conditioning[199] have not supported the case. CONDI-2 (NCT02342522) and ERIC-PPCI (NCT02342522) are multicentre, multinational clinical trials that should provide conclusive results to clarify whether ischaemic conditioning has a role for cardioprotection.

Remote ischaemic preconditioning (RIC), which is defined as repeated brief episodes of ischaemia/reperfusion at a remote site before prolonged ischaemia/reperfusion in a target organ occurs, is proposed as a novel cardioprotective strategy to attenuate reperfusion injury. Cardioprotection by RIC may be mediated by microvesicles. Microvesicles (0.1–1 μm in diameter) are a subset of extracellular vesicles that can be secreted by activated endothelial cells, platelets, erythrocytes and immune cells.[200,201] Currently, microvesicles are studied in the context of cardiovascular biomarkers,[202] in relation to endothelial dysfunction after AMI[203] and in relation to microvascular dysfunction after AMI.[204] However, there is also some evidence that microvesicles, when obtained during RIC, have cardioprotective effects by reducing myocardial infarct size.[205–207] Therefore, the use of RIC-derived microvesicles may be a promising cardioprotective strategy.

CMD is a common complication of AMI, presenting an unmet therapeutic need. Moving forward, we advocate targeted approaches to identify patients who might respond to effective therapy.[208] Use of stratified medicine, coupling novel diagnostics to stratify at-risk patients for targeted therapy, holds future promise.[208] Therapeutic hypothermia during primary PCI may be an option. The EUROpean Intracoronary Cooling Evaluation in Patients With ST-elevation Myocardial Infarction (EURO-ICE) is currently investigating the feasibility, safety and efficacy of localized cooling of the ischaemic area-at-risk by infusion of chilled saline into the infarct-related LAD in patients with anterior STEMI (NCT03447834).

6. Conclusion

In conclusion, reperfusion after STEMI can lead to incremental detrimental effects to the coronary microvasculature. This phenomenon has been described by multiple terms, including no-reflow, MVO, MVI, reperfusion injury, and IMH. All aforementioned pathophysiological mechanisms taken together, we recommend to use the term post-ischaemic CMD as an umbrella term unless specific knowledge on the pathophysiological substrate is available. Post-ischaemic CMD can be predicted using invasive intracoronary measurements such as IMR, HMR, RRR, IHDVPS, Pzf, and visualized using LGE imaging and T2* maps on CMR. Traditional cardiovascular risk factors do not seem to contribute to the development of post-ischaemic CMD. Although multiple clinical trials aimed to target post-ischaemic CMD, none of them were consistently able to reduce the extent of CMD. Targeting coronary microvascular endothelial cells, thereby aiming to prevent vascular leakage and IMH may be a new promising cardioprotective strategy. However, at present, CMD still constitutes an unmet therapeutic need.

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